

# Improved Human Pancreatic Islet Isolation for a Prospective Cohort Study of Islet Transplantation vs Best Medical Therapy in Type 1 Diabetes Mellitus

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**Hypothesis:** A local multiorgan donor pancreas procurement program can provide a source for optimized isolation of purified viable islets for transplantation into patients with type 1 diabetes mellitus receiving best medical therapy.

**Design:** Prospective before-after cohort study.

**Setting:** Tertiary referral center.

**Patients:** Glycemic control was assessed in 10 patients with diabetes-induced renal dysfunction who were enrolled in a best medical therapy program and then crossed over to islet transplantation.

**Interventions:** Thirty human pancreata were retrieved from local multiorgan donors and consecutively processed with intraductal collagenase perfusion, continuous digestion, and density gradient purification (group 1, n=9) or similarly processed but impure tissue fractions cultured in vitro and then repurified to retrieve additional islets (group 2, n=21). Islets were implanted by percutaneous portal embolization, providing more than 10 000 islet equivalents (IE) per kilogram of body weight (infusions from 1-3 donors per patient) under cover of antithymocyte globulin, sirolimus, or mycophenolate mofetil and tacrolimus.

**Main Outcome Measures:** Islet yields, purity, and cell viability (caspase 3, terminal deoxynucleotidyl transferase-mediated biotin-deoxyuridine 5-triphosphate nick-end labeling stain, and insulin secretion in vitro) were com-

pared. In patients, monitored metabolic parameters were C-peptide secretion, insulin requirements, glycemic excursion, and hemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>).

**Results:** For group 1 vs group 2, no differences were observed in pancreas age (43 vs 44 years), cold storage (5 vs 4 hours), or weight (73 vs 82 g). Group 2 yielded 453 690 IE vs 214 109 IE in group 1 ( $P=.002$ ). Grafts contained 50% or more endocrine cells in both groups. No difference occurred in cell viability or insulin secretion. Islets from 90% of group 2 pancreata met release criteria for transplantation. C-peptide secretion was detected in all recipients and persisted with a median follow-up to 12 months (range, 6-21 months) after full islet transplantation. Daily insulin dependence was reversed in all patients for at least 3 months. Five patients resumed small insulin doses. Compared with the best care program, all patients had improved metabolic stability. The mean  $\pm$  SE HbA<sub>1c</sub> level at entry into the study was  $7.8\% \pm 0.5\%$ , and this decreased to  $6.9\% \pm 0.2\%$  after best care ( $P=.38$ ) and further to  $6.2\% \pm 0.2\%$  at 6 months after transplantation ( $P=.002$  vs entry;  $P=.15$  vs best care; analysis of variance).

**Conclusions:** Local pancreas donor retrieval with islet isolation and culture conditioning enabled an offer of islets for transplantation for 90% of consecutively processed pancreata. Isolated islets secreted insulin during prolonged follow-up after implantation into patients, yielding metabolic control comparable with that achieved by best medical therapy.

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**I** NTEREST HAS BEEN RENEWED IN HUMAN islet transplantation for type 1 diabetes mellitus. Recent progress with improved islet isolation, avoidance of glucocorticoid immunosuppression, and provision of adequate islet mass (>10 000 islet equivalents [IE] per kilogram of body weight) from multiple donors has yielded improved islet function in recipients with type 1 diabetes with hypoglycemic unawareness.<sup>1</sup> Despite this advance, significant challenges

remain to obtain sufficient islets. Where established whole-organ pancreas transplantation programs exist, significant numbers of pancreata do not qualify for whole-organ transplantation, and recent data

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suggest that these could be retrieved and subjected to islet isolation to expand a transplantation program for diabetes.<sup>2</sup>

We hypothesized that an effective program of islet transplantation could be established on the strengths of an existing pancreas procurement program that provides for whole-pancreas transplantation. This hypothesis is supported by studies that show that local organ donor procurement programs are the first strength in achieving good-quality human islet isolation.<sup>3</sup> Local donation significantly reduces hypothermic cold storage time that elapses before the pancreas is subjected to islet isolation, thus optimizing the separation of islets from the exocrine and ductal cells of the pancreas.

According to several investigators, more promising success in islet transplantation has been observed in patients who received islet transplants alone.<sup>1,2,4</sup> Many of these recipients had received conventional medical treatment for “brittle” diabetes or hypoglycemic unawareness. It is unknown if transplantation of islets alone may also be an attractive alternative for patients with type 1 diabetes who receive best medical therapy. Best medical therapy is defined as the control of blood glucose with intensive insulin therapy, blood lipids with statins, blood pressure with antihypertensive agents, and renal protection with angiotensin-converting enzyme (ACE) inhibitors. Despite these stringent measures for medical therapy, progressive microvascular complications from type 1 diabetes still occur. In this study, we report on the initiation of a novel protocol for human islet isolation from our local pancreas organ procurement program, in which islet isolation is optimized by repurifying impure tissue fractions saved during the initial pancreas processing. We also report on long-term glycemic control in a cohort of 10 patients who received pancreatic islet transplants after initial treatment in the best medical therapy program.

## METHODS

### EXPERIMENTAL DESIGN

Specific aims of this research were to improve recovery of high-quality human pancreatic islets from individual pancreata and to compare the glycemic control in a cohort of patients with type 1 diabetes who received best medical therapy with their glycemic control after islet transplantation. The study was a prospective before-after cohort design in which all patients were initially enrolled in intensive medical therapy. Data in this study were collected for the initial 10 patients, who completed a minimum of 6 months in both programs. They are the first of a larger ongoing cohort of 50 patients to investigate effects on chronic complications. Inclusion criteria for recipients included a target population of men and women older than 20 years and younger than 60 years with type 1 diabetes confirmed by negative C-peptide levels ( $<0.222$  ng/mL [ $<0.074$  nmol/L]) for more than 5 years, who had elevated microalbuminuria, diabetic retinopathy, a creatinine clearance of more than 70 mL/min (1.17 mL/s), and signed informed consent. Exclusion criteria were body mass index (calculated as weight in kilograms divided by the square of height in meters) greater than 30, previous organ transplantation, active heart disease, planned pregnancy, active infection, malignancy, and lack of compliance. Sample size specification for the study was calculated based on a type I error of .05 and a type II error of .1 for equivalence testing. A consecutive sampling method was performed for the first patients who met inclusion and exclusion criteria. The study was

approved by the research ethics board of the University of British Columbia, Vancouver, in March 2003.

### PANCREAS RETRIEVAL

Human pancreata were obtained (with consent) from adult heart-beating cadaver organ donors through the organ procurement program of the British Columbia Transplant Society, Vancouver. Thirty consecutive human pancreata were retrieved using an identical protocol to that for the whole-organ pancreas transplantation. This includes en bloc dissection with the “no-touch” technique and hypothermic in situ vascular perfusion with University of Wisconsin (UW) solution, with care being taken to avoid elevated portal pressure. Immediate surface cooling of the pancreas was achieved with 4°C ice slush in the lesser omental sac. Pancreata were transported at 4°C in sterile containers to the Ike Barber Human Islet Transplant Laboratory at Vancouver General Hospital, Vancouver, British Columbia.

### ISLET ISOLATION

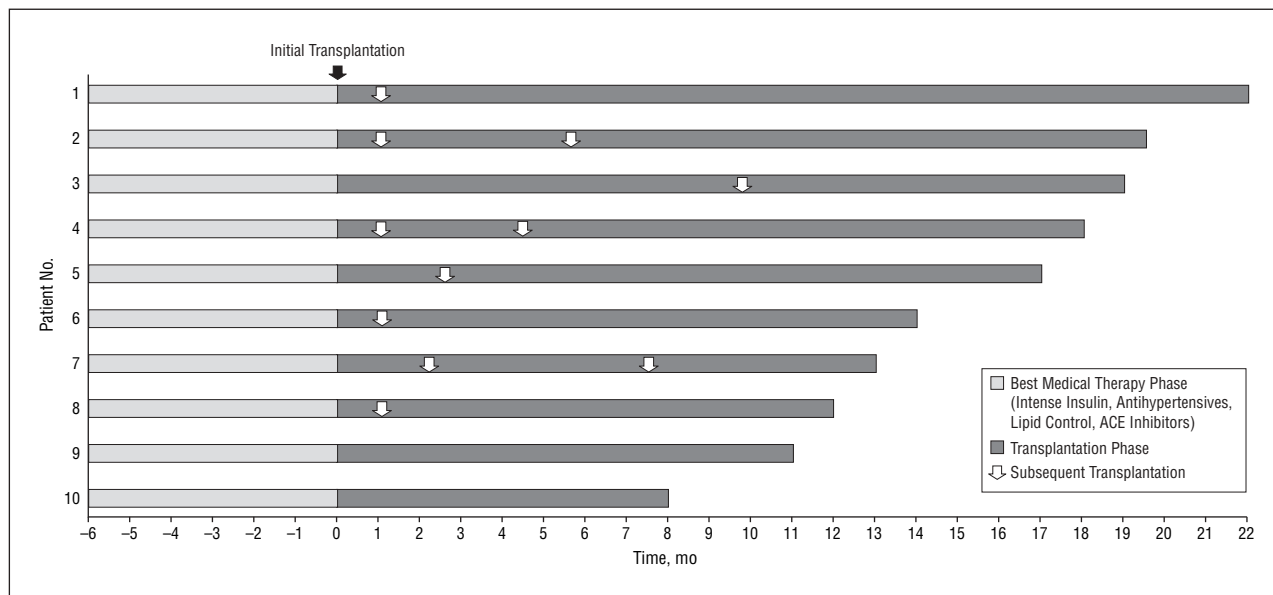
Consecutive pancreata were subjected to islet isolation.<sup>3</sup> The group 1 (n=9) pancreata were processed with collagenase (Liberase HI; Roche Applied Science, Indianapolis, Ind) perfused via the ducts, continuous chamber digestion, and continuous density gradient purification using the Ficoll-Hypaque technique. Group 2 (n=21) pancreata were similarly processed, but additional islets were retrieved by repurifying impure tissue fractions that remained after the initial purification. To accomplish this, the impure tissue fractions were subjected to in vitro culture at 22°C under conditions of 95% air and 5% carbon dioxide in 15-cm tissue culture plates that contained 35 mL of Connaught Medical Research Labs–based media (catalog No. 99-785-cv; MediaTech Inc, Herndon, Va). Cultures were maintained for 12 to 36 hours before the repurification protocol. In all instances, purified islets were subjected to in vitro culture for 12 to 36 hours before transplantation into humans.

### ISLET CHARACTERIZATION

Islet samples were collected in duplicate, counted, and sized for standard yields of islets equivalent to 150  $\mu$ m.<sup>5</sup> Samples were subjected to glucose challenge in vitro to detect insulin secretion, and results were expressed as a ratio of stimulated to basal insulin release.<sup>5</sup> Islet quality control assessment included endotoxin assay by enzyme-linked immunosorbent assay, bacterial cultures, characterization of purity by cell composition,<sup>6</sup> and detection of apoptosis by assay for caspase 3 activity (Cp32 Fluorometric Assay Kit; BioVision Inc, Mountain View, Calif) and terminal deoxynucleotidyl transferase–mediated biotin-deoxyuridine 5-triphosphate nick-end labeling (TUNEL) stain (APO-BRDU Kit; BioSource International, Camarillo, Calif).

### BEST MEDICAL THERAPY AND ISLET TRANSPLANTATION

The indication for patient enrollment in this study was some degree of end-organ damage induced by type 1 diabetes, in particular, early evidence of renal dysfunction defined as elevated microalbuminuria ( $>300$  mg of albumin in 24 hours). The aim was to provide some advantage to all patients with either best medical therapy or islet transplantation. Although hypoglycemic unawareness existed to some extent in 1 patient, this was not a criterion for enrollment in the present study. Persons who were entered into the study complied with protocols during a 3-month run-in period of participation in the University of British Columbia’s best medical therapy program. Best medical



**Figure 1.** Experimental design showing study phases of best medical therapy and follow-up after transplantation of islets into patients with type 1 diabetes mellitus, including subsequent transplantations (when necessary). ACE indicates angiotensin-converting enzyme.

therapy included intensive blood glucose management with insulin, blood pressure control, blood lipid control, and use of ACE inhibitors or angiotensin II receptor antagonists for renal protection. Glycemic control targets were met according to guidelines published by the Diabetes Control and Complications Trial<sup>7</sup> and the clinical practice recommendation of the American Diabetes Association.<sup>8</sup> Patients contacted study nurses twice monthly by telephone or fax for insulin dose adjustment. Blood pressure was treated to targets of less than 130/80 mm Hg to prevent progression of renal disease.<sup>8</sup> Lipid levels were checked every 6 months and treated with statins to reduce predicted 10-year risk of coronary artery disease in diabetes.<sup>9</sup> Elevated microalbuminuria was detected, and an ACE inhibitor (ramipril) was administered in doses up to 10 mg/d.<sup>10</sup>

Patients (5 men and 5 women; mean  $\pm$  SD age, 44.1  $\pm$  7.6 years; mean  $\pm$  SD body mass index, 24.7  $\pm$  1.8) participated for a mean  $\pm$  SD of 15.4  $\pm$  5.3 months in the best medical therapy program and then received islet transplantation (**Figure 1**). The first 2 patients received islets isolated from group 1 pancreata, whereas the subsequent 8 received islets isolated from group 2 pancreata. Patients were transferred to the interventional radiology suite. With the patients under local anesthesia with 1% lidocaine, a 4F catheter (Cook Diagnostic and Interventional Products, Bloomington, Ind) was advanced percutaneously into the liver with fluoroscopic cannulation of the main portal vein. Islets were infused under gravity from each donor while monitoring portal pressure. Catheters were withdrawn, and the hepatic puncture tracks were sealed with Gelfoam (Pharmacia & Upjohn, Kalamazoo, Mich). Patients were observed overnight and discharged home after a mean  $\pm$  SD length of stay of 1.3  $\pm$  0.6 days (range, 1-3 days). Total islet dose exceeding 10 000 IE/kg was provided by 21 total infusions, which necessitated 1 infusion in 2 patients, 3 infusions in 3 patients, and 2 infusions in 5 patients. The final islet dose that was achieved after completed infusions was 13 806  $\pm$  561 IE/kg (n = 10). Immunosuppression consisted of antithymocyte globulin induction (1 mg/kg per day for 5 days) covered with 2 doses of glucocorticoids (125 mg intravenously), followed by introduction of sirolimus or mycophenolate mofetil (n = 2) and tacrolimus. Sirolimus (Rapamune; Wyeth-Ayerst Canada Inc, Markham, Ontario) was given orally to maintain trough blood levels at 5 to 10 ng/mL. Tacrolimus (Fujisawa Canada Inc, Markham, Ontario) was administered to

maintain trough concentration in the blood at 5 to 10 ng/mL. For subsequent infusions of islets, the induction was with the interleukin 2 receptor blocker basiliximab (2 doses of 20 mg).

## PATIENT MONITORING

Patients monitored their glucose levels before and after transplantation using memory capillary glucose meters. The fluctuations of glucose concentrations in each patient were calculated as the mean of the differences in the major fluctuations in high and low glucose values during multiple 24-hour periods and expressed as the mean amplitude of glycemic excursions (MAGE).<sup>11</sup> C-peptide levels and insulin requirements were compared during medical therapy and after transplantation. Hemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>) concentration was assessed at entry to best care, 6 months before transplantation (while receiving best care), and 6 months after transplantation. Duration of follow-up from transplantation in this report was defined as the time since the full (>10 000 IE/kg) islet dose was delivered (12  $\pm$  5 months; median, 12 months; range, 6-21 months). Intensive follow-up was provided by teams of nurses and physicians in the University of British Columbia Diabetes Best Care and Solid Organ Transplant clinical programs.

## DATA ANALYSIS

Data are presented as mean  $\pm$  SE unless otherwise stated. Donor variables, islet yields, insulin secretion, and caspase 3 activity were compared with the *t* test for paired data. Patient follow-up data for blood glucose, insulin requirements, and HbA<sub>1c</sub> concentrations were compared using repeated-measures analysis of variance (ANOVA) with a Bonferroni adjustment of the stated level of statistical significance for multiple comparisons of 3 groups.

## RESULTS

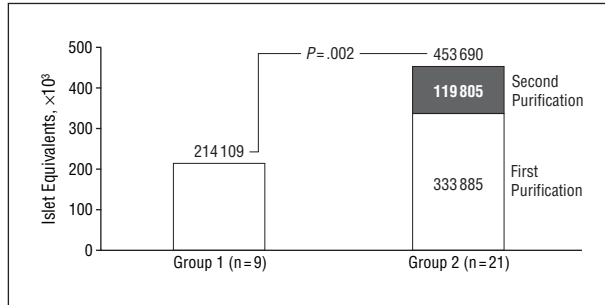
### PANCREAS DONATION AND ISLET YIELDS

**Table 1** gives the pancreatic donor age, cold storage time, and pancreas weight. There were no statistically signifi-

**Table 1. Characteristics of Donor Pancreata Subjected to Consecutive Islet Isolations\***

Group	Donor Age, y	Cold Storage, h:s	Pancreas Weight, g
1 (n = 9)	43.4 ± 7.6	5:19 ± 0:55	73.3 ± 7.7
2 (n = 21)	44.2 ± 3.1	4:04 ± 0:29	81.9 ± 5.4

\*Data are presented as mean ± SE.



**Figure 2.** Postpurification yields. Shading indicates the increased yield after repurification.

cant differences between these 2 groups of donors. A comparison of purified islet yields from group 1 (n=9) and group 2 (n=21) pancreata is shown in **Figure 2**. A total of 214 109 ± 53 184 IE were obtained from the pancreata with single-stage purification, and this was not significantly different from the initial purification of the second group of pancreata, which yielded 333 885 ± 37 576 IE. The addition of a secondary purification significantly improved the overall yields for group 2 to 453 690 ± 48 145 IE ( $P = .002$  vs group 1). Corresponding data in IE per gram of pancreas showed a yield of 2933 IE/g with single purification. The addition of secondary purification significantly increased overall yields to 5533 IE/g.

#### GRAFT CHARACTERIZATION

The concentration of endotoxin in isolated islet preparations was 0.14 ± 0.02 endotoxin units/mL, which was well below the acceptable range. No instance of microbiologic contamination was detected. Insulin release in vitro for the 2 groups of islets revealed a stimulation index of 2.7 ± 0.5 for group 1 vs 3.7 ± 0.8 for group 2 ( $P = .28$ ). Cellular composition of the grafts is given in **Table 2**. The overall proportion of β-cells in group 1 was 35.8% ± 5.7% vs 31.1% ± 4.1% in group 2. A slightly greater proportion of cells bearing ductal (cytokeratin 19) and acinar phenotypes was observed in group 2. Islet cell caspase 3 activity (fluorometric units) as an index of apoptosis was 183 ± 35 in islets after initial purification, which remained unchanged after secondary purification from the same donors at 179 ± 33 ( $P = .93$ ). The TUNEL staining of purified islets showed no difference between primary vs secondary purification.

#### ISLET YIELD FOR TRANSPLANTATION AFTER SECONDARY PURIFICATION

**Figure 3** shows islet yields from the consecutive series of 21 isolations performed with secondary purification. The

data demonstrate that secondary purification augmented the islet yield in all of these 21 isolations. The threshold for islet yield to meet release criteria for transplantation was 250 000 IE. In 19 of the 21 isolations, this threshold was met or exceeded, and in 7 instances this threshold was enabled as a result of the secondary purification protocol. Thus, the overall offer of islets for transplantation was 90% of these consecutively processed pancreata.

#### INSULIN THERAPY, GLYCEMIC CONTROL, AND C-PEPTIDE LEVEL

**Figure 4** summarizes mean 24-hour blood glucose levels and insulin doses during phases of best care, after initial islet transplantation, and after a full islet dose was transplanted. During best care, patients required 0.53 ± 0.6 U/kg per day of insulin. After the first transplantation, insulin doses decreased to 0.19 ± 0.06 U/kg per day ( $P < .001$ ) and after full islet doses, all 10 patients stopped taking insulin completely. Glucose levels remained unchanged at 140.54 ± 0.54 mg/dL (7.8 ± 0.3 mmol/L), 142.34 ± 7.21 mg/dL (7.9 ± 0.4 mmol/L), and 136.94 ± 3.60 mg/dL (7.6 ± 0.2 mmol/L), respectively, during these 3 phases ( $P = .85, .57, \text{ and } .43$ , respectively). Five patients (1 of the 2 who received group 1 islets and 4 of the 8 who received group 2 islets) have continued not to take insulin for 6 to 21 months; the other 5 returned to taking some insulin after further follow-up periods of 3 to 9 months (0.18 U/kg per day). Some of these latter patients preferred a lifestyle choice of enjoying a less restricted diet, for which they compensated by injecting insulin on some days.

C-peptide levels immediately rose from undetectable levels (<0.222 ng/mL [ $<0.074$  nmol/L] in our assay) to levels of 0.601 to 2.40 ng/mL (0.2–0.8 nmol/L) after transplantation and remained persistently elevated during the follow-up of all 10 patients. **Figure 5A** demonstrates C-peptide concentrations throughout the follow-up period for all insulin-independent patients, and **Figure 5B** demonstrates corresponding levels for all patients who returned to some insulin therapy. There was sustained C-peptide secretion at similar levels in both of these groups whether or not they required insulin.

As an index of metabolic lability, the mean amplitude of glycemic excursion scores was calculated, demonstrating values of 7.8 ± 0.5 for best medical care and improving to 3.5 ± 1.3 for the transplantation phase ( $P < .001$ ; paired  $t$  test). This improved stability was reflected by the observation that no patient has had problems of hypoglycemia since the transplantation.

Assessment of HbA<sub>1c</sub> levels is demonstrated in **Figure 6**. Comparison of HbA<sub>1c</sub> concentrations before best care (7.8% ± 0.5%), during best care (6.9% ± 0.2%), and 6 months after islet transplantation (6.2% ± 0.2%) shows significant reduction in HbA<sub>1c</sub> levels. Islet transplantation achieved the best results compared with entry into the study ( $P = .002$ ; ANOVA), but results between best care and transplantation were similar ( $P = .15$ ; ANOVA).

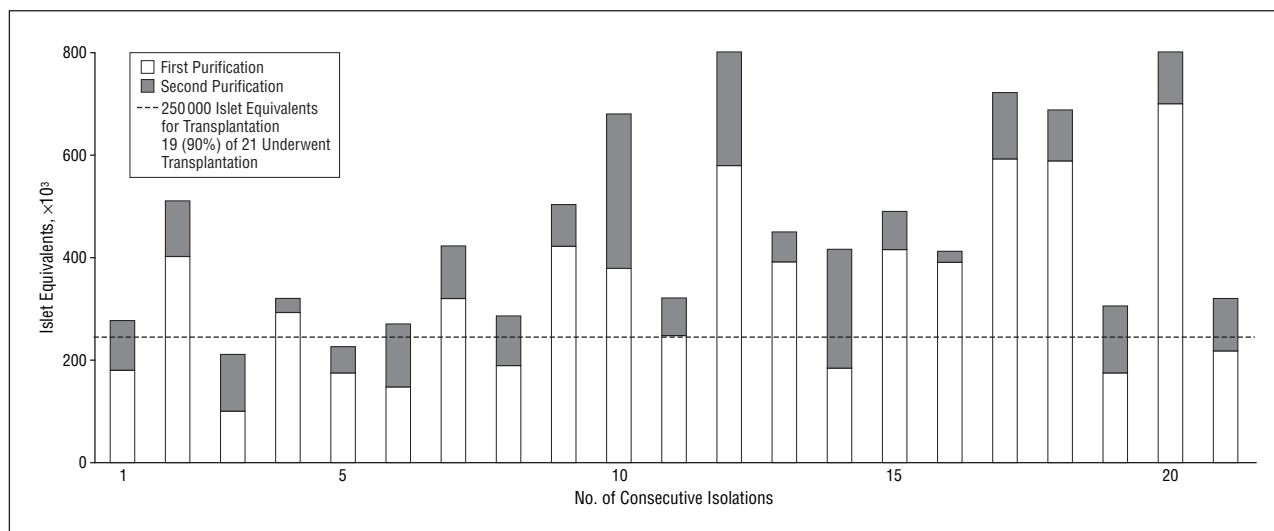
#### COMPLICATIONS

Thirty-eight percent of the 21 infusions were followed by minor degrees of abdominal pain and 15% by nau-

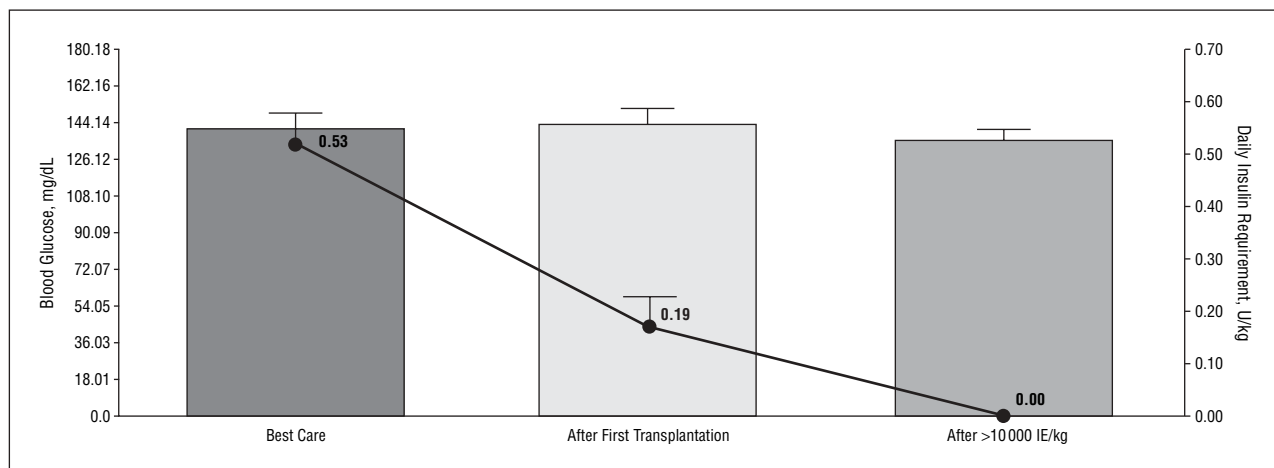
**Table 2. Cellular Composition Analysis of Islet Grafts\***

Group	Ductal Cytokeratin 19	Acinar	$\beta$	$\alpha$	Polypeptide	$\Delta\delta$
1 (n = 6)	15.9 $\pm$ 4.7	20.7 $\pm$ 4.2	35.8 $\pm$ 5.7	13 $\pm$ 2.1	4 $\pm$ 1	3 $\pm$ 1
2 (n = 10)	24.3 $\pm$ 3.4	26.5 $\pm$ 2.7	31.1 $\pm$ 4.1	12.9 $\pm$ 3.3	2.8 $\pm$ .6	2.4 $\pm$ 0.6

\*Data are presented as percentages in mean  $\pm$  SE.



**Figure 3.** Islet yield from consecutive isolations with secondary purification.



**Figure 4.** Daily glucose levels and insulin requirements in 10 patients who were able to stop insulin therapy after islet transplantation. To convert blood glucose to millimoles per liter, multiply by 0.0555. IE indicates islet equivalents; error bars, SE.

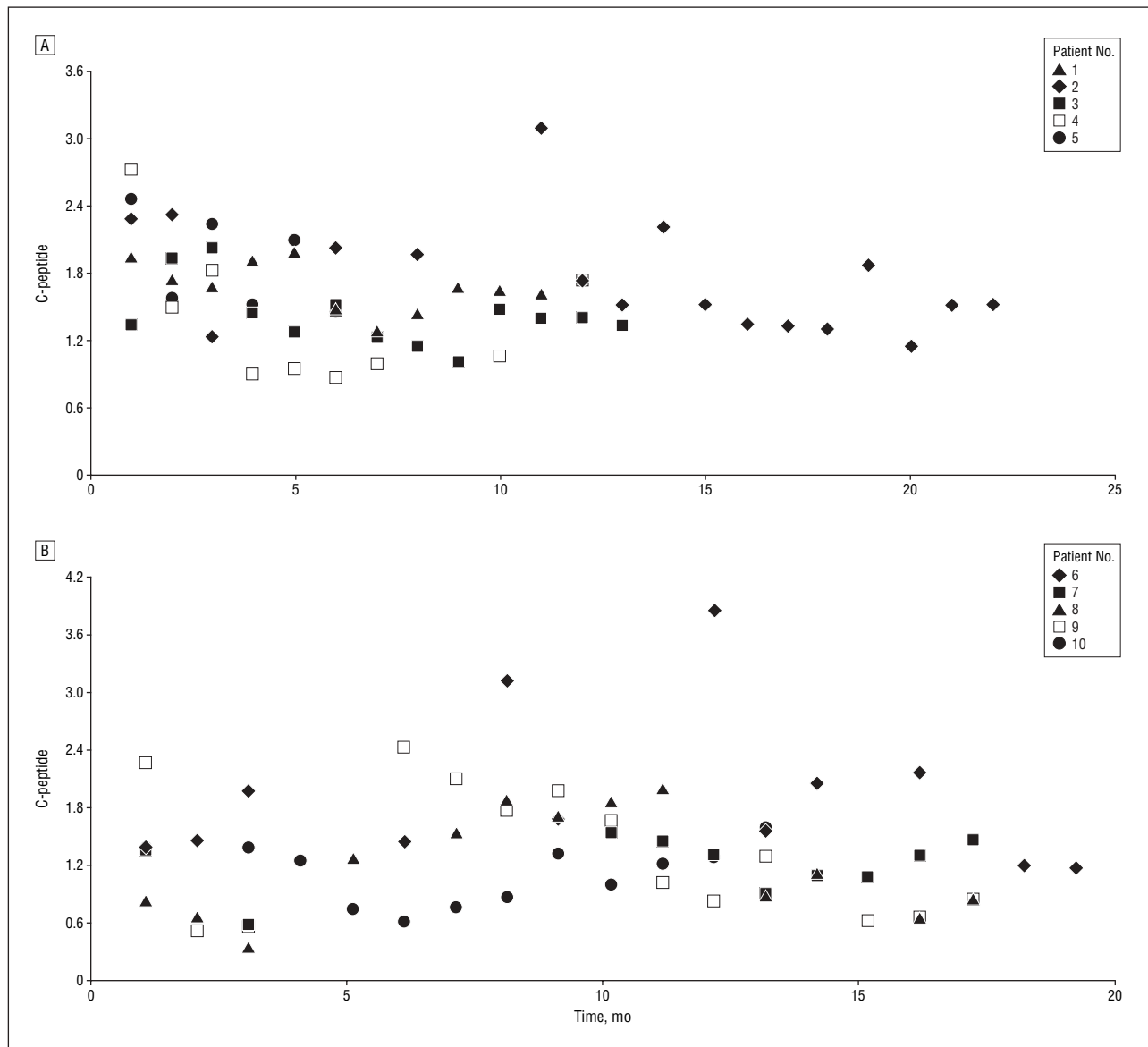
sea. After 3 islet infusions (2 initial and 1 second), post-procedure fluid was detected on ultrasonography, accompanied by a decrease in hemoglobin consistent with bleeding. No blood transfusions and no intervention were required. One of these patients had been receiving acetylsalicylic acid, which was withdrawn until he showed no further decrease in hemoglobin level. For all infusions, portal pressures before infusion were 11.7  $\pm$  3.2 mm Hg, and this increased to 14.0  $\pm$  2.4 mm Hg after infusion ( $P = .005$ ; paired  $t$  test).

All patients who received islet infusions had immunosuppression-related adverse effects of mouth ulcer-

ation ( $n = 10$ ) and neutropenia ( $n = 10$ ). For these patients, the mouth ulcers responded to decreased doses or discontinuation ( $n = 2$ ) of sirolimus therapy with conversion to mycophenolate mofetil. These patients retained stable islet function.

#### COMMENT

The modification to the islet isolation protocol in the current study appears promising. The culture-conditioning protocol added significant islet yields without compro-



**Figure 5.** Posttransplantation C-peptide levels for 10 patients who received isolated islets. A, C-peptide level vs time in patients not receiving insulin during the entire study period. B, C-peptide level vs time in patients who returned to some insulin therapy during prolonged follow-up. To convert C-peptide to nanomoles per liter, multiply by 0.333.

mising islet viability. Although the primary purification protocol tended to have higher yields in the group 2 pancreata processed, this did not appear to be due to differences in the donor characteristics. To exclude the possibility of a type II error, accounting for the (insignificant) trend to higher yields in group 2, we examined the pre-purification yields and identified no differences in this parameter, suggesting that similar numbers of islets were available for purification in both groups. This tends to agree with the group 2 data, which demonstrate an augment of approximately 30% of islet yields when repurification is performed after culture conditioning.

The ability to offer more than 90% of isolated islet grafts agrees with earlier published data that a local retrieval program with short cold storage time is an effective program for providing islets for transplantation. The current data compare favorably with earlier published observations on rates for islets provided from successful

isolations that have been reported as less than 50%.<sup>12</sup> These data should encourage local donor programs to apply these protocols to offer islets for clinical islet transplant programs.

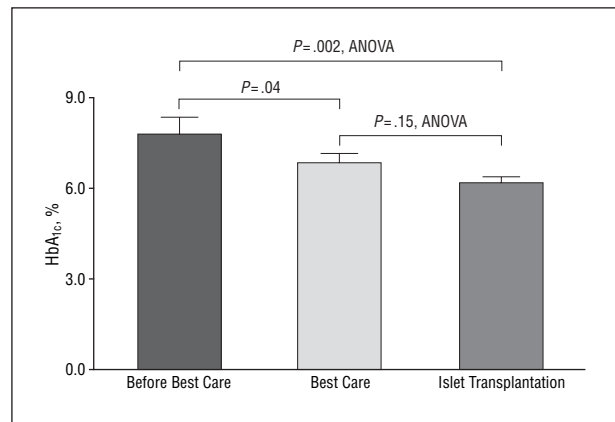
Despite the improvements in islet isolation demonstrated in these studies, patients continued to require large doses of islets. This high islet mass requirement exceeded published data of earlier studies<sup>13</sup> and did not appear to be completely mitigated by glucocorticoid-free protocols. Contributing factors may be related to adverse effects of immunosuppressive drugs on islet engraftment,<sup>14</sup> and more recent data suggest that prothrombotic activity in the portal venous system has an adverse effect on early islet engraftment.<sup>15</sup> One of our recipients had detectable portal vein thrombosis on ultrasonography. He had initial insulin independence, but a delayed need for intermittent insulin was observed after 7 months.

Several investigators have recently reported experience with islet transplantation. Following a report of improved success with the Edmonton protocol in 2000,<sup>1</sup> additional promising reports of islet transplant alone appeared from several centers.<sup>2,4,16</sup> A multicenter trial of pancreatic islet transplantation in patients who followed the Edmonton protocol has been organized via the Immune Tolerance Network, which suggests success at experienced centers but less success in others, and the final data are still pending.<sup>17</sup> Critical analysis of the data from these studies reveals that significant improvement in the rate of insulin independence after isolated pancreatic islet transplantation is attributable to improved islet isolation, glucocorticoid-free immunosuppression, a transplant mass of more than 10 000 IE/kg, and selection of patients with brittle diabetes who have low body mass.

Longer-term follow-up suggests that the initial rate of insulin independence decreases after several months, particularly after the first year. Initial deterioration of function was observed in patients who followed the Edmonton protocol.<sup>18</sup> In a more recent report<sup>19</sup> of 11 transplant recipients with complete long-term data more than 1 year after transplantation, 6 resumed insulin therapy more than 12 months after transplantation. Similar observations have been made by other groups.<sup>2,4,20</sup> In the current study, the islets have also been susceptible to reduced function with time, with patients returning to insulin therapy despite ongoing insulin secretion. The duration of graft follow-up was based on time elapsed after the full dose of islets was administered, which may account for the apparent difference in success rates of insulin independence, since the earlier reports of follow-up began with the first transplantation.

The reasons for delayed decline of function are unclear but may include adverse effects of immunosuppressive drugs on islet function. In the current study, we used antithymocyte globulin, which can induce islet toxicity from cytokine release. We also used a brief dose of corticosteroids to counteract the unpleasant cytokine release effects (fever and myalgia). Both of these agents represent a departure from the protocol reported by Edmonton.<sup>1</sup> Delayed deterioration of an initially marginally engrafted islet mass may be due to an inhospitable intraportal transplantation site. Data from a large pre-clinical animal model previously characterized delayed failure of pure intraportal autologous islets, which was not observed in the splenic site.<sup>21</sup>

Results from the current study compare favorably with collective data recently reported by other investigators.<sup>2,4,20</sup> In a poster report at the 20th International Transplantation Society Congress, September 5-10, 2004, the Collaborative Islet Transplant Registry identified insulin independence in 57.9% of recipients 12 months after their last infusion (B. J. Hering, MD, oral communication, September 6, 2004; www.citregistry.org). The current study contrasts with the earlier studies, since recipients had higher body masses and hypoglycemic unawareness was not a sole indication for transplantation. Longer follow-up will be necessary to determine if this therapy can reduce nephropathy, retinal damage, and endothelial dysfunction better than medical therapy alone. A critical analysis of the



**Figure 6.** Hemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>) levels before and after islet transplantation. ANOVA indicates analysis of variance; error bars, SE.

glucose values in the current study shows improved glycemic excursions, but HbA<sub>1c</sub> remains marginally high at 6.2%, suggesting that caution must be taken to conclude that islet transplantation will prevent microvascular complications on the basis of improved glycemic control alone.

In summary, a strategy for culture conditioning of isolated pancreatic tissue obtained in a local organ donor program provides improved quantities of islets to support clinical islet transplantation. The current study demonstrates that these islets function for prolonged periods after intraportal transplantation into recipients with type 1 diabetes who entered a best medical therapy program, including insulin independence. The long-term follow-up data of the recipients demonstrate that some patients resume insulin therapy despite insulin secretion, a challenge reported by other groups. These data stress the need to redouble efforts to identify factors that may induce and sustain long-term islet function so that this therapy can be evaluated to prevent the long-term complications of diabetes.

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## DISCUSSION

Susan Orloff, MD, Portland, Ore: Thank you for the privilege of opening this discussion of another fine manuscript written by my colleague, Dr Garth Warnock, and his coworkers, as you know, describing the results comparing islet transplantation using multiorgan donors, either with standard collagenase perfusion or the standard perfusion technique plus repurification of impure tissue fractions cultured in vitro. They looked at islet yield, purity, cell viability, insulin secretion, and success of either method of islet isolation and transplantation compared to best medical therapy.

The incidence of diabetes is predicted to increase significantly in the next decade. It already affects 130 million people worldwide. Despite the efficacy of insulin therapy, the devastating secondary complications, including retinopathy, nephropathy, cardiovascular disease, and neuropathy, can shorten the life expectancy by as much as one third. Even though it has

been demonstrated by the Diabetes Control and Complications Trial that tight control of blood glucoses with intensive insulin therapy significantly lowers the level of glycosylated hemoglobin and minimizes the progression of secondary complications, it does not abrogate these complications, and it also results in a significantly higher risk of the complications of severe hypoglycemic reaction, including seizure and coma.

These data underscore the importance of developing better treatment strategies, such as islet transplantation. The concept of transplanting extracts of pancreas in patients with diabetes is over a century old, with the first known report occurring in 1894. In the 1980s, reports of successful allogenic islet cell transplantation in patients with type 1 diabetes with the use of conventional immunosuppression and purified human islets from deceased donors began to appear. But internationally, the overall rates of success were reported to be less than 10%.

In 2000 Shapiro and his colleagues, including Dr Warnock, in Edmonton reported 100% success in 7 patients with modification of the immunosuppressive regimen and multiple infusions of islets from different donors to achieve normal glucose levels and independence from exogenous insulin. However, Edmonton's success has been tough to duplicate elsewhere.

Today we heard from Dr Warnock some very promising results, and I commend you and your colleagues on your efforts and success. However, we will anxiously await your longer-term follow-up of your patients as the current study goes out only 6 to 21 months posttransplantation of the full greater than 10 000-islet equivalents-per-kilogram dose.

I have a few questions. There are a number of successful islet transplant centers, including Minnesota, Miami, and I believe your former institution, Edmonton, that use the perfluorocarbon-based 2-layer method of cold storage to minimize the effect of cold ischemia, and they report an increased islet yield and functional success over static UW preservation. This method has also enabled expansion from the donor pool to include longer ischemia times greater than 10 hours, marginalized pancreata from non-heart-beating donors, and pancreata from older aged donors. You used the static UW solution for transport of your pancreata to the isolation laboratory, and I am wondering if you can comment on why you don't use the perfluorocarbon-based 2-layer method.

In your exclusion criteria, you exclude patients with cardiovascular disease, and you mentioned active heart disease. How is this defined? Do you exclude patients who have had a recent or remote coronary bypass graft and have no symptoms, or are these patients included as possible candidates for islet cell transplantation?

The experience with islet autotransplantation after total pancreatectomy suggests that if ischemia and immune reactivity could be circumvented, fewer islets are required to induce and maintain normoglycemia. You report on 2 patients who only required 1 infusion to reach the dose exceeding 10 000 islet equivalents per kilogram. Did you note any difference in characteristics of the donors, the pancreata, the procurement procedures, or the cold ischemic times in these 2 patients? In addition, you showed total yield on one of your grafts of 214 million IE in the first purification in group 1 who undergo the standard islet cell processing and in group 2 who undergo 2 purifications. In the first purification, the yield is about 334 million IE. Although this is not statistically significant, it does appear to represent a fairly large difference, and I am wondering how you can account for the differences in islet yield.

In the patients who required more than 1 islet infusion to reach the greater than 10 000 islet equivalents per kilogram, did the timing of the next infusion affect the outcome in terms of insulin independence? Also, other than perhaps differences in diet, what was unique to those 5 patients who required some



insulin at 3- to 9-months follow-up after islet cell transplantation? You did show that there were sustained C-peptide secretions at similar levels in the group that did not require insulin compared to the group that did, but at a glance it looks like the levels were lower in the group that required insulin. Have you looked at titers of islet cell antigens and glutamic acid decarboxylase (GAD) antibodies in these patients, suggesting recurrence of the autoimmune response?

In your current protocol, which I actually didn't see in your slide presentation but in the manuscript, you mentioned that there is a minor departure from the Edmonton protocol where you give 2 doses of glucocorticoid, 125 mg. Albeit this is a very small exposure, I am wondering if this has a negative impact on the islets.

Can you comment on whether you think that the intrahepatic site is the best site for islet infusion given the 10% incidence of hepatic bleeding due to the procedure reported in the literature in addition to the high exposure of islets to toxins, including immunosuppressive medications that enter the portal vein, and the rare risk of portal vein thrombosis? Given that the alternative sites, such as omentum or peritoneal cavity, would avoid these issues, in addition, they would require overall less islet mass by avoiding the roughly 50% loss of islets during the purification process. What are your thoughts?

Finally, in this era of organ shortage, there is a significant motivation to search for new sources of transplantable islets. What are your thoughts about the potential for adult and embryonic pluripotent adult and embryonic stem cells, which are expandable in culture and have a potential to give rise to all cell types in the body, including islets, to be used as islet replacement tissue with the possibility of unlimited quantities to be generated? I am only aware of this in *in vitro* studies as well as in mice.

This paper is another very important contribution by Dr Warnock and his colleagues to the evolution of a treatment strategy that has the potential to improve the lives of a vast number of patients and represents the efforts of a highly focused and skilled team. This group undoubtedly has been the leading contributor to this goal, and I want to thank you for sharing your excellent results with us.

**William H. Marks, MD, Seattle, Wash:** I just want to dovetail a question to Dr Orloff's. When you look at the 5 recipients who returned to insulin therapy vs those who remained insulin free, did you find there was a difference in the percentage of the rescued islets that they received? Also, in your response, Dr Orloff, you referred to the culture being central to an effective isolation mechanism. Other groups have also identified a culture step as being an important part of obtaining viable islets. I wonder if you would briefly comment on what you believe to be the survival advantage derived by islets from the culture process.

**Nancy L. Ascher, MD, PHD, San Francisco, Calif:** I was wondering how your group goes about distributing islets vs solid-organ pancreas transplants. I think the audience may not be aware that, in fact, patients who would be candidates for solid-organ transplants are actually competing with those patients who could be candidates for islets. I was wondering how your group makes that distinction.

In addition, as we think about islet transplant outcome vs best practice, I wonder if you don't need a third group to compare and that is the patients who undergo solid-organ transplantations.

**Dr Warnock:** Thank you very much, Dr Orloff, and thanks to the other discussants for your very thoughtful comments. Dr Orloff, that was a very thoughtful review of some of the aspects of islet transplantation that I think helps us to really understand this area much more thoroughly. I will take your comments and questions in order.

You have asked us about comparison with the groups who are using a perfluorochemical, 2-layer pancreas storage. We didn't embark upon this at the time this study started, since the data were just beginning to emerge from the research at that time. Since our short cold storage time here is 4 to 5 hours, our laboratory team is available on call, the retrievals come from within the province, and the pancreas gets delivered to the laboratory right away, we didn't believe that we needed to really set this up. However, I agree with your comment that if one wants to optimize the situation where the logistics of transport require storage for over 8 hours, the data seem to suggest that perfluorochemical, 2-layer storage would be helpful.

You asked about exclusion of patients with active heart disease: this is defined as those persons with symptomatic angina pectoris who have positive MIBI [technetium Tc 99m sestamibi] scanning and certainly when they enter the best medical therapy program, they do receive clinical and MIBI screening, and if medical care can be optimized or stabilized with intervention or coronary artery bypass grafting, then they can become candidates for islet transplants.

You asked about the situation where 2 patients only required a single infusion, and we have indeed had a positive experience of being able to offer single-donor islets. I think that when we look at the dose of islets, one has to keep in mind that many programs select individuals for very lean body mass. They may get a lower islet dose, but it allows them to offer the critical islet mass much more effectively. We don't do that because these individuals have come forward through a screened best medical program and so we take all comers, providing the body mass index is not over 30.

In terms of the question you had about some differences you observed in the number of islets in group 1 and group 2 with a solitary purification, it's a good point. There can be differences in collagenase lots, even though investigators have seen more promising uniformity of lots, reducing variability. There was a shift in the collagenase used at that time. But to address this, we have looked at the prepurification islet yields and certainly didn't see any difference there, so I think our data really do reflect the augmented improvement from the repurification as demonstrated with the consecutive series processed with repurification.

In terms of the patients who had more than one infusion, to get more than 10 000 per kilogram body weight, we have not observed the increment of C-peptide secretion after multiple infusions that was expected. So there is no question in our minds that if you can do this right and get the right dose in there the first time, much the better. I think any step to go to single-donor infusion or even multidonor infusion at one time at the very first time point is the best objective.

You have asked us about those individuals who had returned back to some insulin therapy and whether that might be due to recurrent autoimmunity. Unfortunately, we don't have a way of tracking immune injury. The hopes and expectations that anti-GAD antibodies might discern autoimmunity haven't been proved due to lack of specificity: antibodies can increase whether patients are on insulin or not. Other criteria that our laboratory is evaluating are expression of tolerogenicity or microarray analysis in serum, and this might allow us to distinguish those subsets of individuals who have auto- or alloimmune-mediated vs tolerogenic response.

One of your questions was why some doses of steroids were given. We went to the T-cell-depleting regimen here, in contrast with the Edmonton protocol, to minimize any immune islet injury. These patients actually get quite an uncomfortable response to the antithymocyte globulin (ATG), and we felt the steroids gave them very good symptomatic relief of that. It is possible that those small doses may have had an effect, but in argument with that, some who have had the best long-term function had steroids. To minimize this, now we like to pretreat the re-

ipients before the islet infusion goes in, providing the ATG along with a small dose of steroids before the infusion.

You have wondered if the intrahepatic site is best for islet infusion. It has allowed our radiology interventionalists to come in and do this on an elective basis because of a culture period rather than calling them in at night. But the intraportal site may not be optimal. When you look at preclinical studies in outbred animals, it's very clear that islets in the intraportal site show decline with time in function, and we don't see that in the intrasplenic site. Similarly, the omental pouch works, but unfortunately the islet doses are still quite high.

As far as the growth and development of islets, undoubtedly there is in the pancreas a stem cell, and in some of our preparations there are ductal elements that go in with the graft. Our analysis cannot yet discern if that might be a subset where there is better long-term function, but suffice to say it is extremely controversial in the basic biology literature as to what constitutes a pancreatic stem cell in adult pancreas. We have been very interested in having a developmental biologist work with us to look at the potential that a duct cell in the pancreas with these islets can contribute to better long-term function.

Dr Marks had asked us about the 5 patients who returned to insulin. Was there a difference in the outcome of these because of rescued islets? No, there were no differences in the rescued islets in those groups, but, as I have mentioned, the augment of C-peptide with the additional infusion was not what we expected it to be. What we surmise in that group is that perhaps a reinfusion into the portal circulation might increase the

thrombogenic response with that augmented graft, and in fact there are data now to suggest that.

You have also wondered if culture affects the survival. Certainly, looking at insulin secretion and caspase apoptotic expression in the grafts, it seems that short-term culture does not adversely affect viability. There is a reduced islet number, but that's because the islets do shrink up after culture, and by our standard islet count protocols, would appear to be a reduction in islet mass.

Finally, Dr Ascher has asked us how we allocate organs where there may be a solid-organ or whole-organ pancreas program vs an islet program. Our program does offer whole-organ pancreas transplants to those individuals who have gone on to end-stage diabetic nephropathy, needing a kidney transplant. Those individuals are waitlisted and enter that program. This current study I have shown you is part of an ongoing 50-patient cohort study of patients who have entered best medical therapy with the aim of determining if we can prevent renal failure with a primary outcome of renal function. So there are 2 very separate groups. The 2 programs are complementary, not in competition, which agrees with previous data from the Philadelphia group. Whole-organ transplantation might well be an area that could be a third arm of such a study. I think that at this stage, with the immunosuppressive regimens, the use of calcineurin inhibitors, it makes it very difficult to get those patients into such a treatment protocol because one does need obligate use of those agents at bigger doses and that may adversely affect renal function. So certainly I think in the future, if our immunosuppression would be friendlier, it could be an excellent third arm.