Rapid Publication

Clinical Outcomes and Insulin Secretion After Islet Transplantation With the Edmonton Protocol

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Islet transplantation offers the prospect of good glycemic control without major surgical risks. After our initial report of successful islet transplantation, we now provide further data on 12 type 1 diabetic patients with brittle diabetes or problems with hypoglycemia previous to 1 November 2000. Details of metabolic control, acute complications associated with islet transplantation, and long-term complications related to immunosuppression therapy and diabetes were noted. Insulin secretion, both acute and over 30 min, was determined after intravenous glucose tolerance tests (IVGTTs). The median follow-up was 10.2 months (CI 6.5–17.4), and the longest was 20 months. Glucose control was stable, with pretransplant fasting and meal tolerance–stimulated glucose levels of 12.5 ± 1.9 and 20.0 ± 2.7 mmol/l, respectively, but decreased significantly, with posttransplant levels of 6.3 ± 0.3 and 7.5 ± 0.6 mmol/l, respectively ($P < 0.006$). All patients have sustained insulin production, as evidenced by the most current baseline C-peptide levels 0.66 ± 0.06 nmol/l, increasing to 1.29 ± 0.25 nmol/l 90 min after the meal-tolerance test. The mean HbA_{1c} level decreased from 8.3 ± 0.5% to the current level of 5.8 ± 0.1% ($P < 0.001$). Presently, four patients have normal glucose tolerance, five have impaired glucose tolerance, and three have post–islet transplant diabetes (two of whom need oral hypoglycemic agents and low-dose insulin (<10 U/day). Three patients had a temporary increase in their liver-function tests. One patient had a thrombosis of a peripheral branch of the right portal vein, and two of the early patients had bleeding from the hepatic needle puncture site; but these technical problems were resolved. Two patients had transient vitreous hemorrhages. The two patients with elevated creatinine levels pretransplant had a significant increase in serum creatinine in the long term, although the mean serum creatinine of the group was unchanged. The cholesterol increased in five patients, and lipid-lowering therapy was required for three patients. No patient has developed cytomegalovirus infection or disease, posttransplant lymphoproliferative disorder, malignancies, or serious infection to date. None of the patients have been sensitized to donor antigen. In 11 of the 12 patients, insulin independence was achieved after 9,000 islet equivalents (IEs) per kilogram were transplanted. The acute insulin response and the insulin area under the curve (AUC) after IVGTT were consistently maintained over time. The insulin AUC from the IVGTT correlated to the number of islets transplanted, but more closely correlated when the cold ischemia time was taken into consideration ($r = 0.83$, $P < 0.001$). Islet transplantation has successfully corrected labile type 1 diabetes and problems with hypoglycemia, and our results show persistent insulin secretion. After a minimum of 9,000 IEs per kilogram are provided, insulin independence is usually attained. An elevation of creatinine appears to be a contraindication to this immunosuppressive regimen. For the subjects who had labile type 1 diabetes that was difficult to control, the risk-to-benefit ratio is in favor of islet transplantation.
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Optimal control of diabetes is directed toward a normalization of blood glucose because good glycemic control reduces the long-term risk of diabetes complications. The most definitive study in the field, the Diabetes Control and Complications Trial (1), clearly showed that with intensive insulin therapy, a 2% decrease in HbA_{1c} significantly reduced the risk of microvascular complications. However, this improvement in HbA_{1c} did not result in normalization of the value (median 7%) and was associated with a threefold increased risk of severe hypoglycemia (1), thus providing the incentive to find other methods to achieve excellent glucose control.

Whole-pancreas transplantation has become accepted as an alternative therapy for subjects who are undergoing simultaneous kidney transplants. Although occasionally used as a solitary pancreas transplant, the significant risks associated with whole organ transplantation usually limit its use to co-transplantation with other organs. Pancreas transplantation is still associated with significant morbidity in terms of surgical risk and of cost (e.g., the number of days spent in the hospital), but it is also associated with

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AIR_g, acute insulin response to glucose; AUC, area under the curve, $AUC_{C\text{-}p}$, AUC for C-peptide above baseline; AUCg, AUC for glucose above baseline; AUCi, AUC for insulin above baseline; CMV, cytomegalovirus; HOMA, homeostasis model assessment; IE, islet equivalent; IGT, impaired glucose tolerance; IVGTT, intravenous glucose tolerance test; K_G, the rate of glucose disposal; OGTT, oral glucose tolerance test; MAGE, mean amplitude of glycemic excursion; WBC, white blood cell count.

~86% of graft survival (2,3). Islet transplantation, though much simpler and less costly, has had a poor record of accomplishment in terms of insulin independence. Islet registry data indicate that, at best, 10% of subjects were off insulin at 1 year, although 37% may have continued C-peptide production (4).

The goal of any pancreas or islet transplantation is the achievement of excellent glycemic control with minimal risks (5,6). Islet transplantation, a much less invasive procedure than whole-pancreas transplantation, offers the hope that if performed earlier it will result in excellent glucose control and prevent long-term complications. Clearly, the risks associated with islet transplantation must be less than the risks of leaving the patient with diabetes. For this reason, patients with very brittle diabetes or a severe reduction of hypoglycemic awareness have been selected as suitable candidates for studies of isolated islet transplantation.

Evidently, there are rare short-term risks associated with islet transplantation using intraportal venous catheterization. In addition, there are long-term risks associated with immunosuppressive therapy. Patients and caregivers need to be aware of these risks before the recommendation of islet transplantation is made. Our current protocol uses both long-term sirolimus and low-dose tacrolimus together with short-term dacluzimab and avoidance of the use of corticosteroids; whereas this protocol is novel in this setting, it may have side effects that need to be considered. For the past year, we have had the opportunity to follow 12 consecutive patients who have had successful islet transplantations. This report is a follow-up of our initial communication (7) and provides more detailed information on the progress of these subjects. Although this care is ongoing, the data presented in this article represent results as of 1 November 2000.

RESEARCH DESIGN AND METHODS

Patients with type 1 diabetes (negative C-peptide prestimulation and poststimulation) who had reduced awareness of hypoglycemia, brittle diabetes, or progressive complications, despite optimization of insulin therapy, were considered for this study. A total of 12 consecutive patients (4 women and 8 men) with a mean age of 40 ± 2.7 and a mean duration of diabetes of 29 ± 3.2 years were studied. Pretransplant diabetes complications were recorded, and retinopathy and previous laser therapy were noted (17% had nonproliferative eye disease alone and 42% had previously treated proliferative disease). Nephropathy was recorded as the presence of microalbuminuria (20–299 mg of urinary albumin excretion per day) (75% of subjects), macroproteinuria (≥300 mg of albuminuria excretion per day) (42%), or elevated creatinine (normal ≤115 and ≤125 μmol/l for women and men, respectively) (two subjects). Vascular disease was noted as evidenced by previous (one patient was stable) or symptomatic coronary artery disease, abnormal electrocardiogram, femoral or carotid bruits, intermittent claudication, or diminished peripheral pulses, and the lipid levels were recorded (25% had elevated cholesterol and one patient had hypertriglyceridemia). Peripheral neuropathy was primarily assessed with a neurothesiometer (Scientific Laboratory Supplies, Wilford, England) (8) and with Semmes-Weinstein monofilaments (9) or the presence of severe pain (two patients). Autonomic neuropathy was diagnosed if there was postural hypotension, impotence (four patients), documented bladder or bowel disease attributable to neuropathy, gastroparesis, or gustatory sweating. Control subjects for the metabolic tests (four men and eight women aged 41.7 ± 3.7 years) were confirmed to have normal oral glucose tolerance.

The islets were prepared, and all patients underwent transplantation of islets that were freshly prepared without xenoproteins and treated with a steroid-free immunosuppression regimen, as previously described (7,10–12). Briefly, all patients were given 1 mg/kg dacluzimab every 2 weeks (five doses), sirolimus with trough target levels of 12–15 ng/ml for the first 3 months and 7–10 ng/ml thereafter, and tacrolimus at a target level of 3–6 ng/ml. In addition, patients were given ganciclovir for cytomegalovirus (CMV) prophylaxis, inhaled pentamidine for pneumocystis carinii protection, and vitamin supplements as previously described. The protocol was approved by the Research Ethics Board, University of Alberta, and each person gave written informed consent. All patients required at least two transplantations, and 4 of the 12 patients required three procedures. One patient had two donor pancreases included in the third transplantation procedure.

Metabolic tests. Pretransplantation fasting plasma glucose and HbA_{1c} levels were measured together with memory glucose meter download assessments using a Medisense glucose meter with a Precision Link (Abbott Laboratories, Saint-Laurent, Quebec, Canada). Determination of the mean amplitude of glycemic excursions (MAGE) (13,14), an index of glycemic lability, was performed using seven capillary glucose meter readings a day for two consecutive days. The MAGE was determined pretransplant, midtransplant, and monthly posttransplant. All patients underwent standard-meal tests with blood drawn for glucose and C-peptide at baseline and then at 90 min after consuming 360 ml of Ensure, a liquid meal replacement (Abbott Laboratories). These meal-tolerance tests were scheduled at pretransplant and midtransplant and at 1 week, and 3, 6, 12, and 18 months posttransplant. For all patients posttransplant, renal function in terms of 24-h urine protein and serum creatinine were assessed, and retinopathy, neuropathy, and lipid profiles were documented. Information concerning liver function and other complications was compiled. All patients had an oral glucose tolerance test (OGTT) performed at 6 and 12 months posttransplant, but not pretransplant. Results are provided as the number of months from insulin independence or 1 month after the last transplant, whichever came first, unless otherwise stated.

Subjects underwent an intravenous glucose tolerance test (IVGTT) to assess AIR_g, glucose disposal (K_G), and area under the curve (AUC) for glucose (AUCg), insulin (AUCi), and C-peptide ($AUC_{C\text{-}p}$). The IVGTT was performed at 1, 3, 6, 12, and 18 months posttransplant, with some subjects tested before the first transplant or between transplants. After an overnight fast, an intravenous line was inserted in each antecubital fossa. Two baseline samples were drawn for glucose, insulin, and C-peptide levels over 10 min, and then 50% dextrose (300 mg/kg body wt) was given intravenously over 1 min. Nine samples were drawn over the next 30 min for insulin, C-peptide, and glucose determinations at 3, 4, 5, 7, 10, 15, 20, 25, and 30 min, with 0 time being defined as the beginning of the infusion.

Glucose, insulin, and C-peptide assays. Plasma glucose concentration was determined by the glucose oxidase method (Yellow Springs Instruments, Yellow Springs, OH). Serum insulin was assayed by radioimmunoassay using a commercial kit (Pharmacia, Uppsala, Sweden). The intra-assay and interassay coefficient of variation was <7%, and all samples were measured in duplicate. C-peptide immunoreactivity was measured as described by Faber et al. (15) using a commercial assay (Diagnostic Products, Los Angeles, CA). The lower limit of sensitivity of this assay in our laboratory was 0.35 nmol/l, and the inter- and intra-assay coefficient of variation was <8%. As of October 2000, the C-peptide assay used has been a commercial assay from Diagnostic Systems Laboratory (Webster, TX), with a lower limit of sensitivity of 0.003 nmol/l and a coefficient of variation of <9.5%.

Data analysis. AIR_g was defined as the mean of the values of the samples from the 3rd-, 4th-, and 5th-minute time points above the baseline. K_G was determined from the slope of the natural log of the glucose values on the IVGTT from 10 to 30 min. The AUCg, AUCi, and $AUC_{C\text{-}p}$ were calculated over the full 30 min and represented the area above baseline. We assessed insulin sensitivity using the homeostasis model assessment (HOMA) as an estimate of insulin sensitivity based on fasting glucose and insulin levels (16).

Islet numbers were quantified in terms of islet equivalents (IEs) with the use of a standard islet diameter of 150 μm (17). Islet grafts were also characterized in terms of percent islets of the infusate (purity). An ischemic index was calculated using the number of islets transplanted multiplied by 10^{-3} and divided by the total cold ischemia time (time from cross clamp until time of islet infusion into the patient), so that a transplant of 400,000 IEs with a cold ischemia time of 8 h gives an ischemia index of 50. The ischemic index for each infusate was summed as a total for each patient. An in vitro insulin stimulation index was derived by maintaining some of the islets in culture and exposing them to a glucose challenge (18). These islets were incubated for 24 h at 37°C in CMRL-1066 medium, 10% fetal calf serum, and 25 mmol HEPES buffer. A known number of duplicate aliquots of islets were incubated in both low and high glucose concentration mediums (2.8 and 20 mmol/l), and the amount of insulin released in the high glucose concentration divided by that of the low glucose concentration provided the in vitro insulin stimulation index.

Statistics. Results are expressed as means ± SE or median (25–75% CI), and groups were compared with a Student's t test and a Mann-Whitney U test when equal variance tests failed. Kruskal-Wallis one-way analysis of variance on ranks was used for multiple group comparisons versus control subjects,

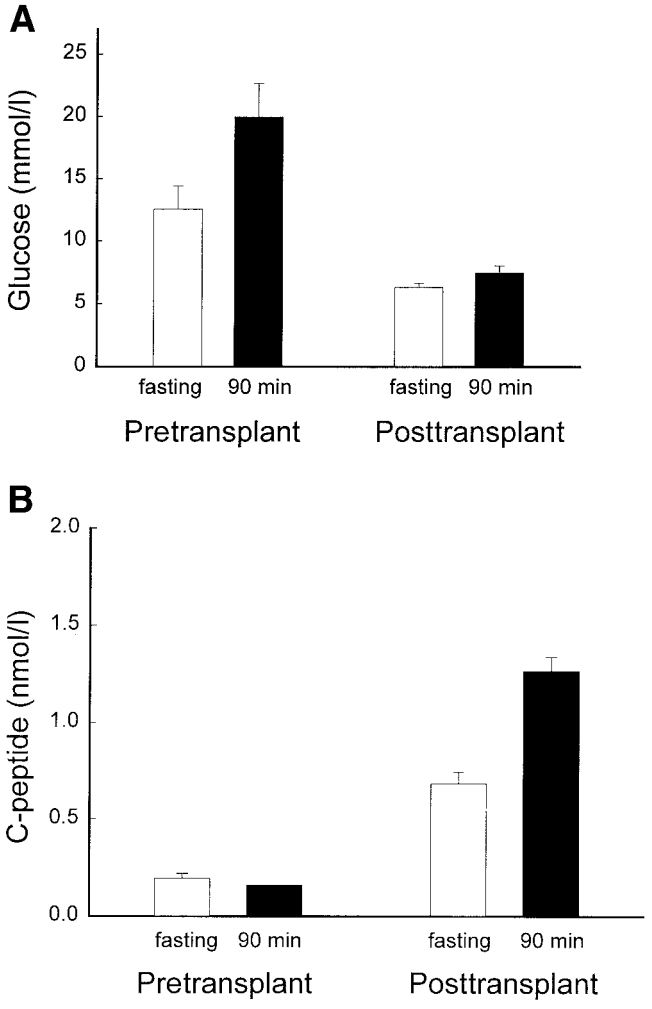

FIG. 1. *A*: Plasma glucose levels derived from the standard-meal tolerance tests in patients pre- and post–islet transplantation. Fasting and stimulated value at 90 min are shown. Values are mean ± SE of the mean. *B*: Plasma C-peptide levels derived from the standard-meal tolerance tests in patients pre– and post–islet transplantation. Fasting and stimulated values at 90 min are shown. Values are means ± SE. Levels pretransplant were below detectability of the assay.

using Dunn's method as appropriate. Statistical tests and linear regressions were assessed using Sigma-Stat (Jandel, San Rafael, CA), and a P value of <0.05 was deemed significant.

RESULTS

Glycemic control. By 1 November 2000, 12 patients had completed islet transplantations; the longest follow-up was 20 months, with a median follow-up of 10.2 months (CI 6.5–17.4) from the first transplant. The glucose levels from the meal-tolerance tests before and after transplant are shown in Fig. 1*A*. The pretransplant fasting and stimulated glucose levels of 12.5 ± 1.9 and 20.0 ± 2.7 mmol/l decreased significantly posttransplant to 6.3 ± 0.3 and 7.5 ± 0.6 mmol/l ($P < 0.006$ and $P < 0.003$, respectively). The OGTT was performed at 12 months in four subjects of whom two were normal, one had impaired glucose tolerance (IGT), and one was in the diabetic range. Four other subjects had the 6-month OGTT performed: one patient was normal, two had IGT, and one was diabetic. Of the remaining four subjects, one had normal glucose levels, two had impaired fasting glucose, and one had diabetes based on fasting glucose levels and meter readings. Thus, at that time, it appeared that four of the subjects had normal glucose tolerance, five had IGT, and three had post–islet transplant diabetes, a form of type 2 diabetes. All had stable glucose levels and continued C-peptide production at a median follow-up of 6 months (CI 1.6–12) (Fig. 1*B*). Two of the three subjects with diabetes are being treated with oral hypoglycemic agents (started at 3 and 6 months after the final transplant, one patient each being treated with metformin and rosiglitazone), and the other patient is being managed on diet alone. In this latter patient, HbA_{1c} has recently increased, and the patient will be started on oral hypoglycemic agents. The two subjects currently on oral hypoglycemic agents are taking low doses of insulin (2–10 U/day), and the insulin dose is being lowered as the oral hypoglycemic agents take effect. Of note is the fact that the three subjects with diabetes are women, and one had some technical problems with the transplantation (vide infra). Two of the IGT subjects have used insulin on rare occasions (less than once a month) in small doses for hyperglycemia associated with intercurrent illness.

HbA_{1c} levels improved in all patients, with pretransplant levels of 8.3 ± 0.5% that decreased to the current level of 5.8 ± 0.1% ($P < 0.001$) at a median of 9 months (CI 4.5–15) (Fig. 2*A*). Eight of the nine subjects have values that are in the normal range, and the three subjects with diabetes have an HbA_{1c} >6.1% (the upper limit of normal); the highest level was 7.1% in the diet-treated subject who is just starting oral hypoglycemic agents. This degree of glycemic control was achieved with no episodes of hypoglycemia, mild or severe. Figure 2*B* shows a range of capillary glucose values encountered over the follow-up period, as derived from the memory glucose meters. The vast majority of the values are in the range of 3.5–7.7 mmol/l. Because few patients have been transplanted for a year or more, there is a relatively small number of values in the extended follow-up period (at 18 months $n = 1$ for glucose meter readings). The stability of the glucose control is also documented in the MAGE results. The MAGE improved when the first transplant was performed and stabilized after the final transplant was performed, and it has remained stable for the duration of follow-up (Fig. 2*C*).

Islet requirements. The mean number of islets transplanted at the first transplant was 363,212 ± 22,918 and at the second was 370,386 ± 39,953 islets. Each patient received a mean of 857,318 ± 43,314 islets (four patients had three procedures). Exogenous insulin requirements dropped substantially after the first transplantation, from a pretransplant dose of 0.56 ± 0.04 to 0.26 ± 0.05 U/kg before the second transplant, $P < 0.001$ (Fig. 3). In 11 of the 12 subjects, when a minimum of 9,000 IE/kg were transplanted, insulin independence was attained (Fig. 3). The one subject who was not off insulin after receiving >9,000 IE/kg had a thrombosis in a peripheral portal vein that did not lead to any long-term consequences, but may have contributed to a partial loss of function of this graft. All patients discontinued insulin after the final transplant at a median of 1 day (CI 0–3.5). The in vitro measures of the transplanted islets showed a percent purity of 67 ± 3% and an insulin stimulation index of 5.88 ± 1.0.

Acute complications. As mentioned in our original report (7), two patients had bleeding from the liver surface

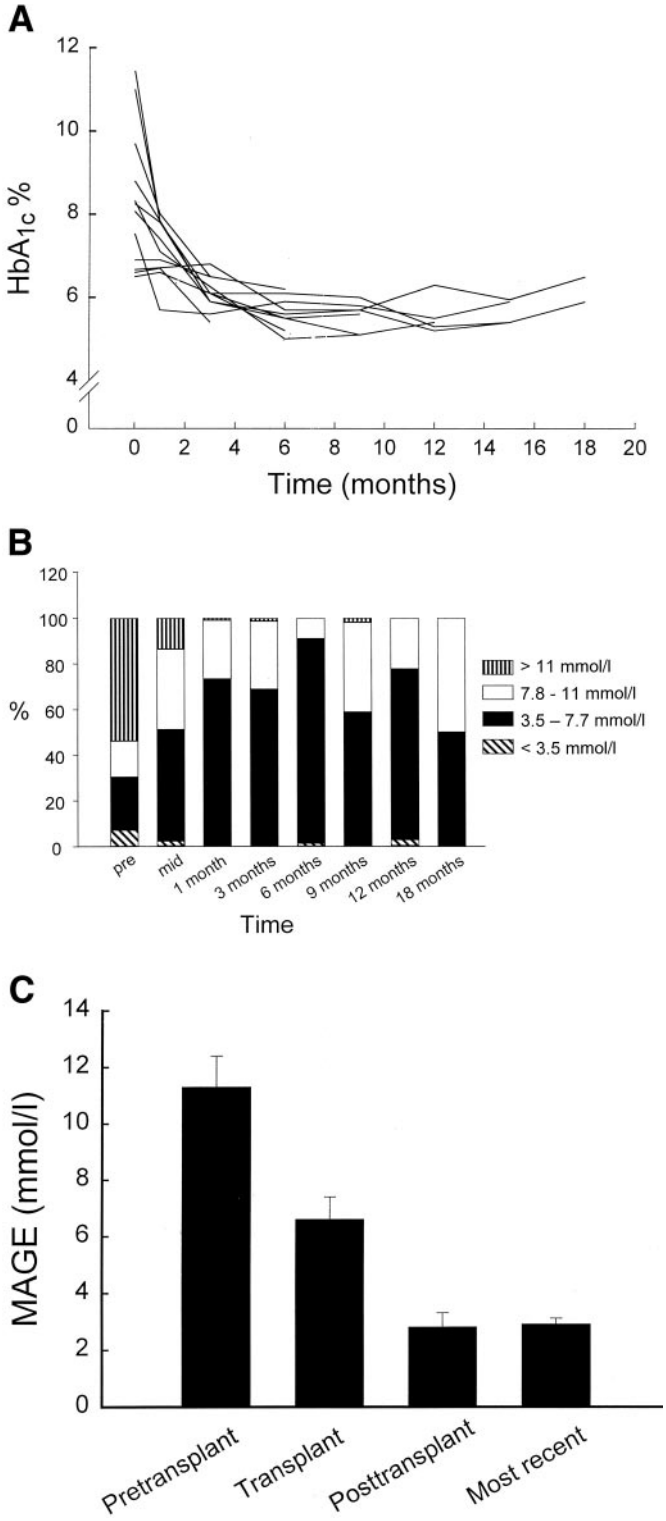

FIG. 2. *A*: HbA_{1c} at 3-month intervals post–islet transplant. All patients showed an improvement in HbA_{1c}. *B*: Capillary glucose values obtained from the memory glucose meter in patients undergoing islet transplantation over time. Values are divided into different ranges and are represented as shown in insert. *C*: MAGE values derived from frequent capillary glucose sampling (seven values/day for two consecutive days) in patients undergoing islet transplantation over time. Values are means ± SE.

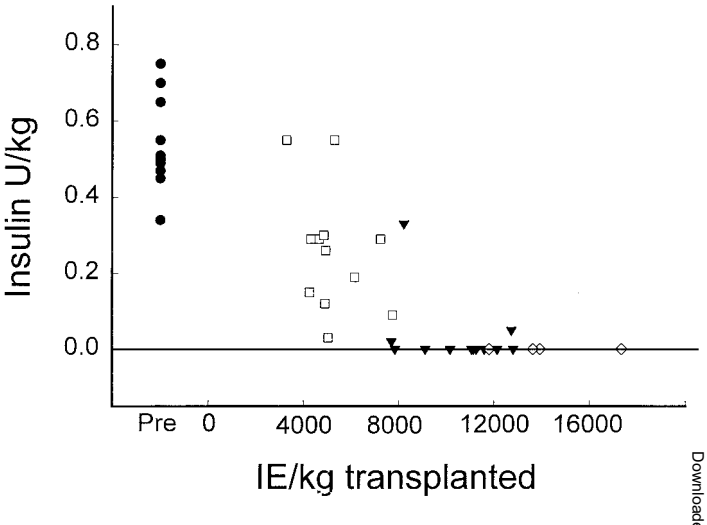

FIG. 3. Daily exogenous insulin use (U/kg) in relation to the number of islets transplanted expressed as IE/kg. Values for pretransplant (●), after the first transplant (□), after the second transplant (▼), and after the third transplant (◊). All patients became insulin independent when adequate islet numbers were provided.

after the transplant procedure that required blood transfusion. However, those were some of the initial patients, and since we introduced a marked reduction in the dose of heparin given with the islet infusion (500 IU or one-tenth the original dose) and since we instituted the use of hemostatic Gelfoam (Pharmacia and Upjohn, Mississauga, Ontario, Canada) after the hepatic catheterization, this problem has not been encountered in any more procedures (a total 28 performed). Three-quarters of the subjects had no significant changes in any liver function tests, but three patients had increased (>2× the upper limit of normal) liver function tests with a mean peak aspartate aminotransferase of 201 ± 57 (normal <40 U/l) and all resolved. In one of these patients, the liver ultrasound showed some increased echogenicity, and a liver biopsy was performed. This biopsy revealed fatty liver, but three islets of Langerhans were identified with a single-pass 18-gauge core (Fig. 4). There was no evidence of inflammatory infiltration in the islet graft. One of the patients with abnormal liver function tests had a thrombosis of a peripheral segmental section of a portal vein. Either the Gelfoam (in this case Gelfoam was used as a liquified preparation rather than a plug) or the fact that this infusion was directly into a minor branch of the portal vein may have caused this. The patient was anticoagulated for 2 months and recovered without problems, and all subsequent infusions were into the main portal vein. Most patients had some abdominal discomfort of varying intensity after the procedure. This may be have been related to some peritoneal irritation, but it subsided spontaneously in all cases.

Long-term complications. The majority of the patients had some pre-existing renal involvement, as evidenced by microalbuminuria (75%) and macroproteinuria (42%), but only two patients had elevated creatinine levels pretransplant (160 and 158 μmol/l [normal range <125] and creatinine clearance values of 0.77 and 0.92 $ml \cdot s^{-1} \cdot 1.73 M^2$, respectively [normal range 1.5–2.3). Both of these patients were believed to have stable renal status, but encountered significant worsening of renal function (creatinine levels increased to 300 and 223 μmol/l, respectively). Overall, the group's mean serum creatinine did not change (prevalue 99 ± 14 μmol/l and current level 95 ± 16

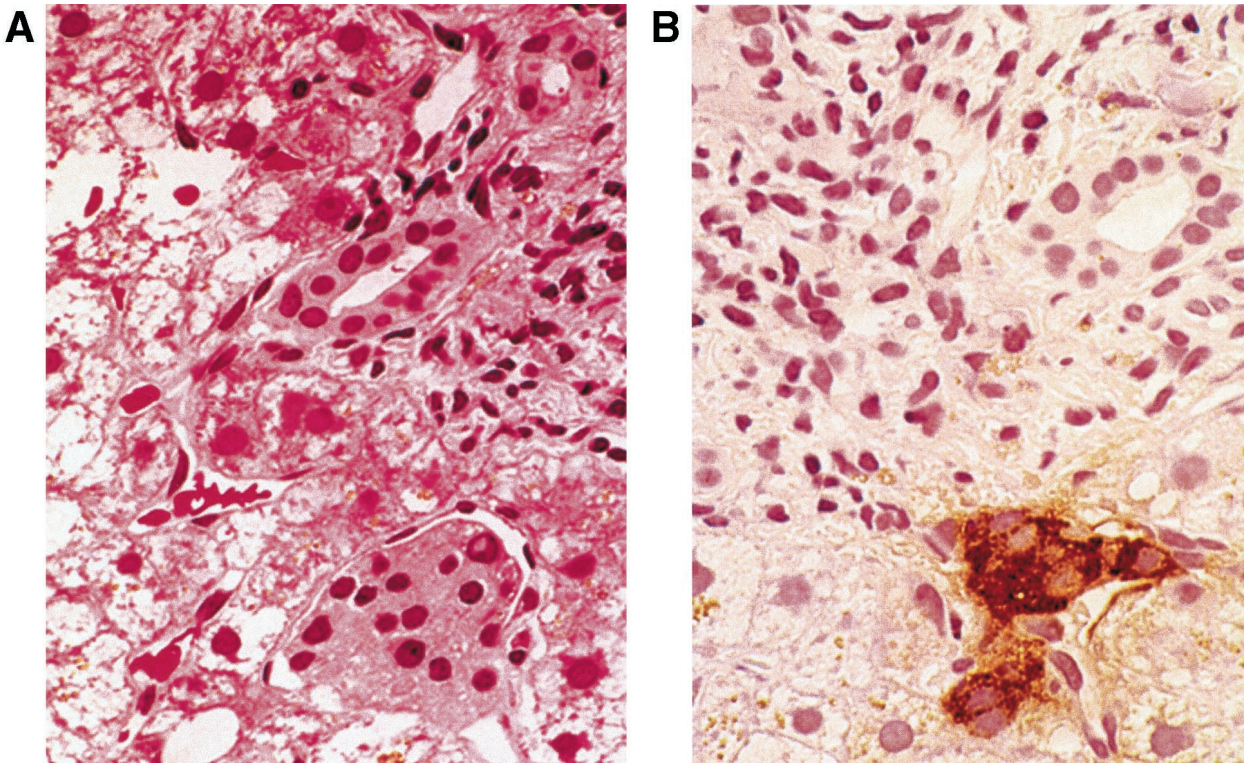

FIG. 4. Liver biopsy from patient with an islet transplant. *A*: The islet cell is in close proximity to the portal triad, portal vein, hepatic vein, and bile duct; *B*: Immunohistochemistry staining with insulin antibody to confirm the presence of insulin-containing cells.

μmol/l). In the one patient in whom the serum creatinine had increased to 300 μmol/l, the tacrolimus was tapered, and the serum creatinine decreased to 246 μmol/l. For both patients, we plan to stop the tacrolimus and to use mycophenolate. Another patient had pretransplant proteinuria of 0.3 g/day, and this increased acutely to 0.7 g/day and has since stabilized with an increase in the dose of ACE inhibitor. Seven patients were on ACE-inhibitor therapy pretransplant, and in two of these patients a calcium channel blocker was also being used for blood pressure control. Four patients had an increase in blood pressure that required an increase in antihypertensive therapy.

Triglyceride levels and HDL levels did not change over the course of follow-up. Five patients had an increase in serum cholesterol, and three patients were placed on statin therapy. One patient was on lipid-lowering therapy pretransplant. In all patients, the cholesterol-to-HDL ratio remained <4. The elevated lipids responded to the statin therapy appropriately, with no complications. Five patients had problems with loose bowel movements, but no specific cause was found, and empirical use of cholystyramine has eased this problem in all patients.

In terms of diabetes complications, there was no change in neuropathy as measured by neurothesiometer testing or by pressure filaments. Two patients developed vitreous hemorrhages that settled promptly, required minimal laser photocoagulation, and have not had any residual effect. No other changes in retinopathy were documented. One patient felt that her necrobiosis lipoidica diabeticorum was better. One patient developed a superficial thrombophlebitis for which temporary anticoagulation was used in light of a history of previous deep venous thrombosis several years ago. Mouth ulcers (usually mild and superficial) occurred in 10 of 12 patients, but settled spontaneously in all but one patient who required surgical debridement before resolution. This problem has improved considerably since we began administering the tablet rather than the liquid formulation of sirolimus and since we implemented lower target levels of the drug.

We have not seen any CMV disease or infection, despite the high degrees of mismatching in 8 of 12 subjects (positive donor to negative recipient). No posttransplant lymphoproliferative disorder or malignancy has been detected. There have been no life-threatening infections, and one episode each of pneumonia and cellulitis in separate patients responded to therapy. None of the patients were sensitized to donor antigen, and all patients currently have negative panel-reactive antibodies.

There was a decrease in body weight from 70.5 ± 2.7 kg pretransplant to 66.1 ± 3.1 kg posttransplant ($P < 0.02$). The mean hemoglobin level decreased significantly from 140 ± 4 g/l pretransplant to the current level of 117 ± 5 g/l ($P < 0.001$). The platelet count did not change. The mean white blood cell count (WBC) decreased from 6.9 ± 0.4 10^9/l pretransplant to 4.8 ± 0.4 10^9/l at the latest follow-up ($P < 0.001$). In four patients it decreased to <3.5 for two consecutive values, and drug doses were adjusted when appropriate. If the drug levels were satisfactory, the ganciclovir was held until the WBC recovered and then restarted.

Insulin secretion. Insulin secretion was maintained over the 12 months, as shown in Fig. 5. Two patients have been studied pretransplant. The AIR_g increased minimally midtransplant and then plateaued for the duration of follow-up (Fig. 5*A*). The AIR_g of the subjects was significantly reduced when insulin-independent, at 19% of that of the

nondiabetic subjects, and at midtransplant, it was only 2% of that of the control subjects. In 33 of the 44 IVGTT studies performed, the fasting glucose was ≤6.4 mmol/l at the commencement of the test. K_G was reduced and consistent throughout (Fig. 5*B*). The AUCi was negative pretransplant, present at a low level mid-transplant (7% of control subjects), and then consistently positive post–insulin-independence (Fig. 5*C*). When insulin independent, the AUCi of the subjects was significantly lower, at 36% of that of the control subjects. The $AUC_{C\text{-}p}$ mirrored the AUCi results. Insulin sensitivity, as determined by the HOMA program and the AUC levels for glucose during the 30 min of the IVGTTs, was similar at all time points and not reduced compared with control subjects.

The AUCi correlated with the number of islets transplanted, expressed as total islets (Fig. 6*A*) ($r = 0.59$, $P = 0.004$). The $AUC_{C\text{-}p}$ also was related to the number of islets transplanted ($r = 0.45$, $P = 0.034$), as was the AIR_g ($r = 0.50$, $P = 0.017$). The fasting plasma glucose did not correlate with the AIR_g ($r = -0.40$, $P = 0.064$). To evaluate the parameters important in the preparation of the islets for in vivo function in terms of insulin secretion, we examined the relationship of the purity of the islet preparation in terms of the percent islet cells and the in vitro insulin stimulation index. No relationship was evident with either of these two measures and AIR_g, fasting glucose, or meal tolerance test–stimulated glucose levels. However, there was a stronger correlation between the ischemic index and the AUCi ($r = 0.83$, $P < 0.001$) (Fig. 6*B*). No correlation was evident between the fasting or meal tolerance test–stimulated glucose value and the ischemic index.

DISCUSSION

All patients have been resolved of unstable type 1 diabetes and now have no problems with wide swings of blood glucose or hypoglycemia. A total of 3 of 12 patients have post–islet transplant diabetes, a diabetes characterized by stable glucose levels, persisting endogenous insulin production, and no problems with hypoglycemia. Post–islet transplant diabetes is readily managed with oral hypoglycemic agents and low-dose insulin. The majority of the 12 patients had labile diabetes with marked swings in their glucose levels, as evidenced by the MAGE and frequency of hypoglycemia. These aspects of their diabetes have been completely corrected. One-third of the patients have absolutely normal glucose tolerance, as defined by stringent OGTT criteria (for three patients), and 42% have IGT. The majority of patients continue to have excellent glycemic control off insulin. In the minority of patients whose glucose control has slipped, there have been no problems with hypoglycemia and labile glucose levels, although two of them are taking oral hypoglycemic agents and low-dose insulin. Compared with their initial brittle status or problems with hypoglycemia awareness, these patients still consider this a major improvement.

The most serious adverse event we saw was an increase in the serum creatinine levels in two patients who already had elevated serum creatinine. The majority of patients had some microalbuminuria, and this has not changed, but long-term follow-up will be required to determine whether it has improved. However, in both patients with elevated creatinine levels, the increase in serum creatinine was significant and would prompt the exclusion of people with elevated serum creatinines from future islet transplantation until calcineurin inhibitors (the most likely culprits ([19]) can be avoided. Four of the patients had an increase in blood pressure that prompted an increase in antihypertensive therapy. Undergoing islet transplantation involves a risk/benefit assessment that must be individualized. For the moment, it appears that significant renal involvement, such that the baseline serum creatinine is above normal, involves risks that outweigh benefits.

In terms of the short-term problems, the acute bleeds appear to have been solved by the use of much less heparin and by the use of a solid plug of Gelfoam. The peripheral portal branch vein thrombosis was a technical problem and has not been seen in any of the other 28 cannulations. None of the serious surgical complications that may occur with whole-pancreas transplant were evident in any of these patients, and the procedure was simple and very well tolerated (20). In terms of long-term problems, diarrhea is a concern for five patients, but appears to be responding to cholystyramine. No specific cause has been found as of yet, but it is clearly related to the introduction of immunosuppressive therapy and has been found in other clinical studies for both sirolimus and tacrolimus (21,22).

Common known side effects of sirolimus include hypertension, diarrhea, and hyperlipidemia (23). Other problems can be arthralgias, rash, acne, thrombocyptopenia, leukopenia, hypokalemia, and mucosal herpes (23). Two patients had some pain in the feet that may have been arthralgias, but both settled, and there was no evidence of any arthritis. We did notice a significant mild decrease in the hemoglobin and white cell count, and one patient had a problem with acne. Major side effects of tacrolimus include nephrotoxicity, neurotoxicity, glucose intolerance, and gastrointestinal upset (22). We have had little problems with headaches or tremor, but as previously documented, the two patients with known renal disease had nephrotoxicity and some had bowel disturbance and glucose intolerance. Patients with glucose intolerance did not have higher tacrolimus levels. The daclizumab has not been associated with any major problems, as of yet (24,25).

In terms of diabetes complications, it is too early to say whether there is a sustained long-term benefit from islet transplantation. Presumably, the improved HbA_{1c} levels without the risk of hypoglycemia are beneficial. In the short term, it is of note that 5 of the 12 patients developed an increase in cholesterol. Although treatable, it is clear that we do not want to accentuate the risk of atherosclerosis through islet transplantation. Emerging preliminary data with the use of sirolimus-coated stents in coronary occlusion suggest that sirolimus may retard restenosis, and if this proves to be the case, it could modify the gravity of the dyslipidemic changes (26). Two of the patients developed a vitreous hemorrhage, which is disturbing, but this may be related to the acute improvement of the hyperglycemia and has been seen in other studies (27,28). In both patients, things have settled down, and there are no long-term effects. There has been no change in patients with severe painful neuropathy, but, as evidenced by other

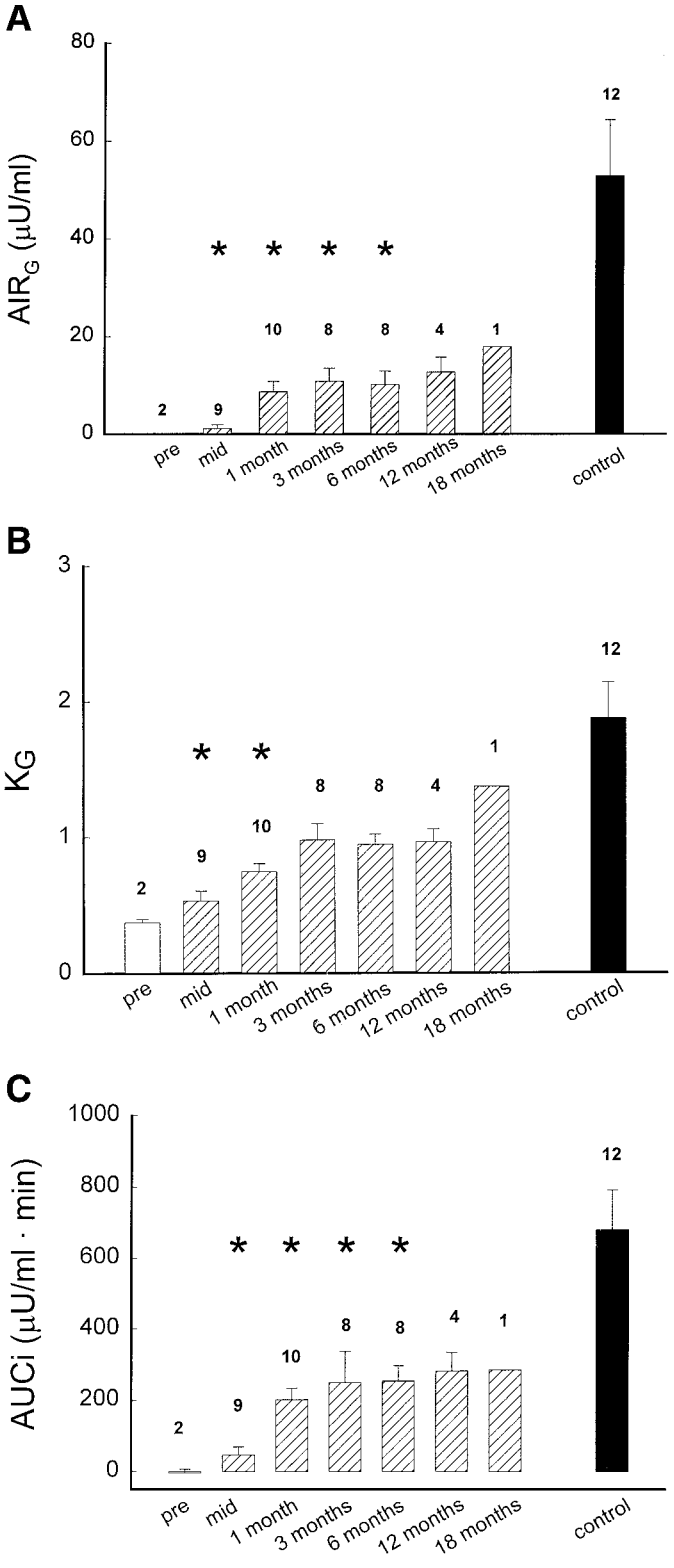

FIG. 5. *A*: AIR$_g$ (microunit/milliliter) over time in subjects pre– and post–islet transplantation, as derived from the IVGTT. Time is described in relation to the islet transplant procedure and "mid" refers to studies performed between the first and last transplant. The number of subjects studied is shown across the top. Mean values ± SE are provided for each time point. Values for mid, 1, 3, and 6 months are significantly less than those for control subjects (*P < 0.05). Pretransplant and 18-month values were not tested because of lack the of numbers. *B*: K_G over time in subjects pre– and post–islet transplant, as derived from the IVGTT. The number of subjects studied is shown across the top. Mean values ± SE are provided for each time point. Values for mid and 1 month are significantly less than those for control subjects (*P < 0.05). Pretransplant and 18-month values were not tested because of the lack of numbers. *C*: AUCi over time in subjects post–islet transplant as derived from the IVGTT. The number of subjects studied is shown across the top. Mean values ± SE are provided for each time point. Values for mid, 1, 3, and 6 months are significantly less than that for control subjects (*P < 0.05). Pretransplant and 18-month values were not tested because of the lack of numbers.

studies, it may take many years before any benefit is realized in this area.

Long-term adequate insulin secretion has been difficult to maintain after islet transplantation, but our results currently show that in the present series we have been successful. Although it took at least two transplants to achieve insulin independence, once it was achieved, it was maintained in the majority of subjects. Some subjects continue to have IGT as evidenced by occasional high values, particularly during an intercurrent illness. However, the data raise some important questions. What is remarkable is that even after the infusion of >850,000 islets, AIR$_g$ and AUCi are still significantly lower than that in nondiabetic subjects. It is thought that a normal pancreas has approximately one million islets (29), and yet, whereas >85% of this mass was transplanted, only 19% of the normal AIR$_g$ and 36% of the normal AUCi was obtained.

The AIR$_g$ data showing such a modest insulin response must be interpreted with some caution; despite the poor AIR$_g$ midtransplant, there was a significant reduction in exogenous insulin requirements. It increased the likelihood that in this setting, the AIR$_g$ is not the best measure of islet mass for these patients, although the AIR$_g$ during the IVGTT was the best measure of insulin secretion in previous studies of auto islet transplantation (30). The poor response at the midtransplant time period may reflect some degranulation of the islet insulin content that has improved by the time the subject has become insulin independent. Insulin response is usually maintained once fasting glucose is <6.4 mmol/l (31), and virtually all of the patients off insulin and only a minority of the midtransplant patients maintained a fasting glucose at this level. However, throughout the months of follow-up, the response remains strikingly reduced. Perhaps the inherent denervation of these islets is a factor, or it may be that the specialized vascularization of endocrine tissue is not recurring. In the normal islet, the capillaries are fenestrated to allow for increased permeability, and if the new vasculature of the transplanted islets lacked this feature, it may be a cause of the blunting of the AIR$_g$ (32,33). The acute insulin response to arginine may prove a better measure of insulin reserve, and we are currently testing this approach. It may take hyperglycemia-potentiated arginine responses or another approach, such as using radionucleotide scanning, to measure viable islet mass in this setting.

The AUCi and the AUC$_{C\text{-}p}$ both showed minimal response midtransplant and substantial improvement after the second transplant, but they are still reduced compared with normal. This may suggest that there is preferential destruction of islets after the first transplant. No matter which measure of insulin reserve we use, the response is less than expected for the number of islets transplanted. A partial pancreatectomy does not usually result in diabetes (34), and yet a single islet transfusion of 354,000 islets was inadequate to relieve a person of the need for insulin. The

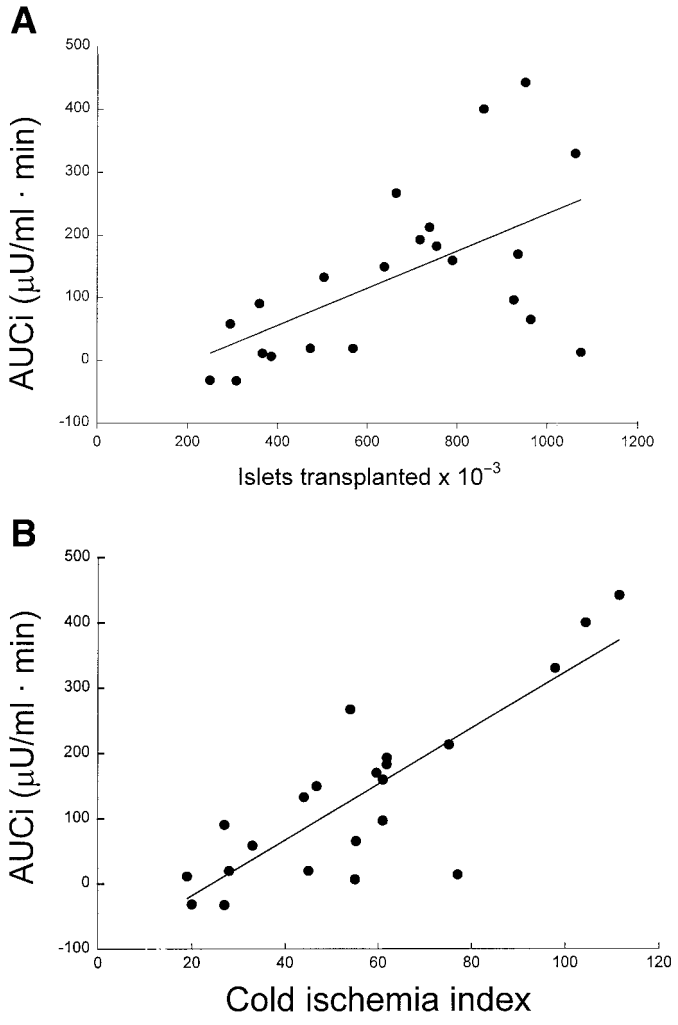

FIG. 6. *A*: Relationship of AUCi as derived from the IVGTT in subjects at midtransplant (n = 10) and at 1 (n = 3) or 3 months (n = 9) post–islet transplant and the number of islets transplanted. Data include results after the first and subsequent transplants; r = 0.587, P = 0.004. *B*: Relationship of AUCi, as derived from the IVGTT in subjects at midtransplant (n = 10) and at 1 (n = 3) or 3 months (n = 9) post–islet transplant and the ischemia index (islet numbers × 10^{-3}/cold ischemia time); r = 0.828, P < 0.001.

reason for this less-than-expected performance is not clear. The counting of islets is an approximation in that a correction factor is used to establish IEs. Although direct counting is commonly used, validation is not complete. Perhaps in time DNA analysis will provide a more accurate method. This pattern suggests that there is a loss of islets after transplantation, and further efforts need to be directed toward improving the survival of all transplanted islets, efforts that may allow successful single-donor–alone islet transplantation.

We had the opportunity to assess whether there was a relationship between the number of islets transplanted and the insulin response to glucose as expressed by AUCi, AIR_g, and $AUC_{C\text{-}p}$. This was confirmed, but no relationship was evident between the number of islets transplanted and the actual fasting plasma glucose or the stimulated glucose level. Other factors play a role in glucose tolerance, and these factors may include not only the destruction of some of the islets that have been transplanted, but also other aspects of glucose control. It does not appear to be an issue of insulin sensitivity, because the HOMA results indicate that no significant insulin resistance was induced by our immunosuppressive regimen and that insulin sensitivity is consistent throughout. As has been previously recognized, it is clear that minimizing the cold ischemia time may be very important in terms of achieving optimal islets per transplantation (35). We did find a stronger correlation between ischemic index and AUCi, AIR_g, and $AUC_{C\text{-}p}$ than any of these measures directly compared with IEs. The ischemic index is a simple concept that attempts to take into account the cold ischemia time for a given islet infusion. If islets are harvested locally, given a cold ischemia time of 5 h, and 300,000 islets are obtained, the cold ischemia index for this infusion is 60 (300,000 × 10^{-3} divided by 5). Likewise, 600,000 islets that have a 10-h cold ischemia time will have the same ischemic index. For two transplantation procedures, we simply added the cold ischemic indices, and it does appear to be a valid number in terms of a significant relationship with AUCi, AIR_g, and $AUC_{C\text{-}p}$. It is unknown whether any techniques are available to improve islets that have been exposed to a lengthy ischemic time. It is conceivable that culture may revive the islets, although the opposite is also possibly true. Certainly, cultured islets in the past have not been noted for their success.

What are the important lessons to learn to further improve the results of islet transplantation? Whereas the Edmonton Protocol appears to be less nephrotoxic than standard cyclosporine and higher-dose tacrolimus-based regimens, even low-dose tacrolimus should be used with caution in the face of significant impairment in baseline renal reserve (as evidenced by an increased serum creatinine level). The further development of calcineurin–inhibitor free regimens remains attractive. The use of sirolimus without cyclosporine and glucocorticoids may still lead to dyslipidemia, but in general, this is less severe than the previous experience and is reflected predominantly by changes to cholesterol rather than triglycerides. Procedural complications related to portal embolization may be avoided by islet infusion into the main portal rather than into a peripheral venous branch, and the use of a solid plug of thrombostatic agent rather than a liquified preparation may reduce the risk of peripheral branch-vein thrombosis. The use of smaller doses of heparin reduces the risk of bleeding from the liver surface after the procedure. The risk of development of severe mouth ulceration with sirolimus is minimized by the use of the tablet rather than the liquid preparation and by lowering the initial target levels of this drug. Finally, it is evident that insulin independence is usually achieved when a minimum of 9,000 IE/kg are provided. Using the measures of insulin secretion available, the insulin reserve midtransplant is very blunted and even with subjects off exogenous insulin, the insulin secretion is less than expected for the number of islets transplanted. Perhaps other measures of surviving islet mass are needed in this situation, as are other mechanisms to enhance islet survival. Insulin secretion is maintained over the follow-up time available. The cold ischemia time may be an important determinant of islet viability in terms of insulin secretion after transplantation.

It is clear from our experience that islet transplantation

is most effective in controlling labile diabetes and effectively protects against unrecognized hypoglycemia in highly selected patients. The degree of metabolic control achieved in most patients is likely to have a positive impact on the advancement of secondary diabetic complications, provided that this can be maintained in the long term, but this remains to be proven. When considering an islet transplant, it is essential that the risk-to-benefit ratio be in favor of the islet transplant. It is evident from these results that in someone who has stable diabetes without any significant complications, this may not be the case. However, in those patients with significant problems with glycemic control, particularly reduced hypoglycemic awareness, or brittle diabetes, the benefits of achieving the stable glucose control that is offered by islet transplantation appears worthwhile, provided that the baseline creatinine level is normal. None of our patients want to stop the immunosuppressive therapy, and all of them consider the transplantation to be worthwhile and beneficial, but it will take further follow-up to determine whether there is a net benefit. The short-term results are certainly encouraging.

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