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Restoration of Glucose Counterregulation by Islet Transplantation in Long-standing Type 1 Diabetes

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Patients with long-standing type 1 diabetes (T1D) may exhibit defective glucose counterregulation and impaired hypoglycemia symptom recognition that substantially increase their risk for experiencing severe hypoglycemia. The purpose of this study was to determine whether intrahepatic islet transplantation improves endogenous glucose production (EGP) in response to hypoglycemia in T1D patients experiencing severe hypoglycemia. We studied longitudinally subjects (n = 12) with ~30 years, disease duration before and 6 months after intrahepatic islet transplantation using stepped hyperinsulinemic-hypoglycemic and paired hyperinsulinemic-euglycemic clamps with infusion of 6,6-$^2H_2$-glucose and compared the results with those from a nondiabetic control group (n = 8). After islet transplantation, HbA_{1c} was normalized, and time spent while hypoglycemic (<70 mg/dL) was nearly abolished as indicated by continuous glucose monitoring. In response to insulin-induced hypoglycemia, C-peptide (absent before transplant) was appropriately suppressed, glucagon secretion was recovered, and epinephrine secretion was improved after transplantation. Corresponding to these hormonal changes, the EGP response to insulin-induced hypoglycemia, which was previously absent, was normalized after transplantation, with a similar effect seen for autonomic symptoms. Because the ability to increase EGP is ultimately required to circumvent the development of hypoglycemia, these results provide evidence that intrahepatic islet transplantation can restore glucose counterregulation in long-standing T1D and support its consideration as treatment for patients with hypoglycemia unawareness experiencing severe hypoglycemia.

Defective glucose counterregulation develops in long-standing type 1 diabetes (T1D) due to progressive impairments in defense mechanisms against a falling plasma glucose concentration in the setting of therapeutic hyperinsulinemia (1). This includes loss of inhibition in endogenous insulin secretion with associated loss of activation in glucagon secretion, which together normally increase endogenous (primarily hepatic) glucose production (EGP); impairment in sympathoadrenal epinephrine secretion, which contributes to EGP; and symptom generation, which leads to a syndrome of hypoglycemia unawareness also known as hypoglycemia-associated autonomic failure (HAAF) (2). Hypoglycemia unawareness in T1D is associated with a 20-fold increased risk for experiencing severe hypoglycemia (3), which itself contributes importantly to increased morbidity (4) and mortality (5).

In cross-sectional studies of long-standing T1D, after intrahepatic islet transplantation, insulin-induced hypoglycemia is associated with normal inhibition of endogenous insulin secretion and either defective or partially restored glucagon secretion, epinephrine secretion, and symptom responses (6–8). We sought to determine whether the recovery of islet responses to hypoglycemia after transplantation together with avoidance of hypoglycemia afforded by functioning islet grafts would reverse HAAF and restore the EGP

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response by studying longitudinally the same patients before and 6 months after transplantation.

RESEARCH DESIGN AND METHODS

Subjects included had long-standing C-peptide–negative T1D complicated by hypoglycemia unawareness and frequent severe hypoglycemic events and underwent islet-alone transplantation (n = 12) as part of the Clinical Islet Transplantation (CIT) Consortium protocols conducted at the University of Pennsylvania. These included all 11 subjects participating in the CIT07 protocol from our institution (9) and 1 of 2 participants who experienced early islet graft failure with the CIT05 protocol that included rituximab for induction and who later underwent retransplantation under an Edmonton protocol (10). The transplant recipients underwent one or two intraportal infusions of islets to achieve insulin independence.

Healthy nondiabetic control subjects (n = 8) were matched for sex, age, and BMI to the T1D subjects. The study protocols were approved by the Institutional Review Board of the University of Pennsylvania, and all subjects gave written informed consent to participate.

Continuous Glucose Monitoring

T1D subjects were evaluated using a 72-h continuous glucose monitoring system (CGMS) (Medtronic MiniMed) before and at 75 days after islet transplantation.

Metabolic Studies

T1D subjects underwent randomized paired hyperinsulinemic-hypoglycemic and -euglycemic clamps before and 6–7 months posttransplantation, each after a 12-h overnight fast from 2000 h. At t = −120 min, a primed (5 mg/kg · fasting plasma glucose in mg/dL/90 for 5 min) continuous (0.05 mg · kg^{-1} · min^{-1} for 355 min) infusion of the stable glucose isotope tracer 6,6-$^2H_2$-glucose (99% enriched) (Cambridge Isotopes Laboratories, Andover, MA) was administered to assess EGP before and during the induction of hyperinsulinemia (11). When necessary overnight to target the baseline blood glucose at 81–115 mg/dL, a low-dose insulin infusion was continued during this period to maintain normoglycemia until t = 0. After baseline blood sampling at −20, −10, and −1 min, at t = 0 min, a continuous infusion of insulin was initiated at 1 mU · kg^{-1} · min^{-1} for 240 min. Subsequently, a variable-rate infusion of 20% glucose was administered to either achieve hourly plasma glucose steps of ~80, 65, 55, and 45 mg/dL for the hypoglycemic clamp or maintain plasma glucose of ~90 mg/dL for the euglycemic clamp (12). To reduce changes in plasma enrichment of 6,6-$^2H_2$-glucose during the clamp, the 20% glucose solution was enriched to ~2.0% with 6,6-$^2H_2$-glucose (11). Plasma glucose was measured every 5 min at bedside with an automated glucose analyzer (YSI 2300; Yellow Springs Instruments) to adjust the glucose infusion rate and achieve the desired concentration. Additional blood samples and an autonomic symptom questionnaire were collected every 20 min and analyzed as previously described (7,8,10). Samples from paired hypoglycemic-euglycemic experiments of each subject pre- and 6 months posttransplant were assayed simultaneously.

Calculations and Statistics

The rate of appearance of glucose during the clamps was calculated using the Steele non–steady-state equation modified for the use of stable isotopes as previously described (10). EGP was calculated from the difference between the rate of appearance of glucose in the plasma and the infusion rate of exogenous glucose. The magnitude of each hormonal, EGP, free fatty acid, and incremental symptom response was assessed as the mean of values obtained during the last 60 min of hypoglycemia.

All data are expressed as mean ± SE. Comparison of results between pre- and posttransplant T1D subjects and between each T1D group and control subjects was performed with paired or unpaired Student t tests or the corresponding nonparametric test as appropriate using Statistica software (StatSoft Inc.). Significance was considered at $P < 0.05$ (two-tailed).

RESULTS

Subject Characteristics and Continuous Glucose Monitoring

The T1D subjects were of comparable sex, age, body weight, and BMI distribution with the nondiabetic control subjects (Table 1). HbA_{1c}, which was elevated before, decreased to nondiabetic levels after islet transplantation ($P < 0.01$) (Table 1). The T1D subjects had ~30 years of disease duration and an insulin requirement of ~0.5 units · kg^{-1} · day^{-1} that was substantially reduced ($P < 0.01$) (Table 1) after receiving 9,648 ± 666 islet equivalents/kg body weight. Seven subjects were insulin independent after one islet infusion, three were insulin independent after two islet infusions, one was insulin dependent while awaiting a second islet infusion, and one remained insulin dependent after two islet infusions. Subjects maintained appropriate levels of tacrolimus and sirolimus (Table 1).

The selection of T1D subjects for the presence of hypoglycemia unawareness, severe hypoglycemia, and marked glycemic lability was reflected in the substantially elevated Clarke score (12), HYPO (hypoglycemia severity) score (13), and lability index (13), respectively, before transplantation (Table 1). After transplantation, the Clarke score of reduced hypoglycemia awareness became negligible, glycemic lability was markedly reduced, and the CGMS demonstrated substantial reductions in mean glucose (164 ± 11 to 121 ± 4 mg/dL, $P < 0.01$), glucose SD (73 ± 6 to 23 ± 4 mg/dL, $P < 0.01$), and time spent hyperglycemic (glucose >180 mg/dL) (38 ± 5 to 4 ± 3%, $P < 0.01$), with essentially no time spent hypoglycemic (glucose <70 mg/dL) (12 ± 2 to 2 ± 1%, $P < 0.01$) (14) (Supplementary Fig. 1).

Insulin and Glucose During the Hypoglycemic and Euglycemic Clamps

The insulin infusion during the hypoglycemic clamp resulted in comparable hyperinsulinemia in the T1D

Table 1—Subject characteristics

	T1D subjects		
	Before islet transplantation	After islet transplantation	Nondiabetic control subjects
Male/female sex (*n*)	5/7	5/7	4/4
Age (years)	45 ± 3	47 ± 3**	44 ± 3
Weight (kg)	71 ± 3	65 ± 3**	77 ± 5
BMI (kg/m^2)	25 ± 1	23 ± 1**	25 ± 1
HbA_{1c} (%)[a]	7.1 ± 0.2	5.6 ± 0.1**	5.5 ± 0.1**
T1D duration (years)	29 ± 4	31 ± 4**	—
Insulin use (units · kg^{-1} · day^{-1})	0.48 ± 0.05	0.06 ± 0.05**	—
IE/kg transplanted	—	9,648 ± 666	—
Tacrolimus (μg/L)	—	4.3 ± 0.4	—
Sirolimus (μg/L)[b]	—	8.4 ± 0.5	—
Clarke score[c]	6.3 ± 0.2	0.4 ± 0.3**	—
HYPO score[d]	2,564 ± 715	ND	—
Lability index[e]	719 ± 67	93 ± 56**	—

Data are mean ± SE. IE/kg, islet equivalent (whereby an islet equivalent approximates a standard islet diameter of 150 μm) transplanted per kilogram of recipient body weight; ND, not done. [a]To convert to mmol/mol, multiply by 10.93 and subtract 23.50. [b]One subject was converted from sirolimus to mycophenolate mofetil as a result of the development of interstitial pneumonia 4 weeks after transplant that subsequently resolved (9). [c]Clarke score of hypoglycemia unawareness (7 = most, 0 = none) (12). [d]HYPO score of hypoglycemia severity developed by Ryan et al. (13). [e]Lability index measure of glycemic lability developed by Ryan et al. (13). **$P < 0.01$ for comparison with T1D subjects before transplantation.

subjects before and after transplantation (99 ± 14 vs. 79 ± 5 μU/mL) and in the control subjects (84 ± 8 μU/mL), which was also not different in any group from the hyperinsulinemia achieved during their respective euglycemic control experiments. During the hypoglycemic clamp, plasma glucose by 60 min was near 80 mg/dL in all three groups and overlapped thereafter during the 65, 55, and 45 mg/dL hourly glucose steps, whereas during the euglycemic clamp, plasma glucose remained between 85 and 90 mg/dL.

Islet Cell Hormonal Responses

Endogenous insulin secretion, as measured by C-peptide, was inhibited by hyperinsulinemia during the euglycemic clamp similarly in both the T1D subjects after transplantation and the control subjects, with identical complete suppression occurring during hypoglycemia (Fig. 1*A* and Table 2). Glucagon was suppressed during hypoglycemia in the T1D subjects before transplantation ($P < 0.01$ vs. control subjects) to levels not different from that under euglycemic conditions, whereas glucagon increased

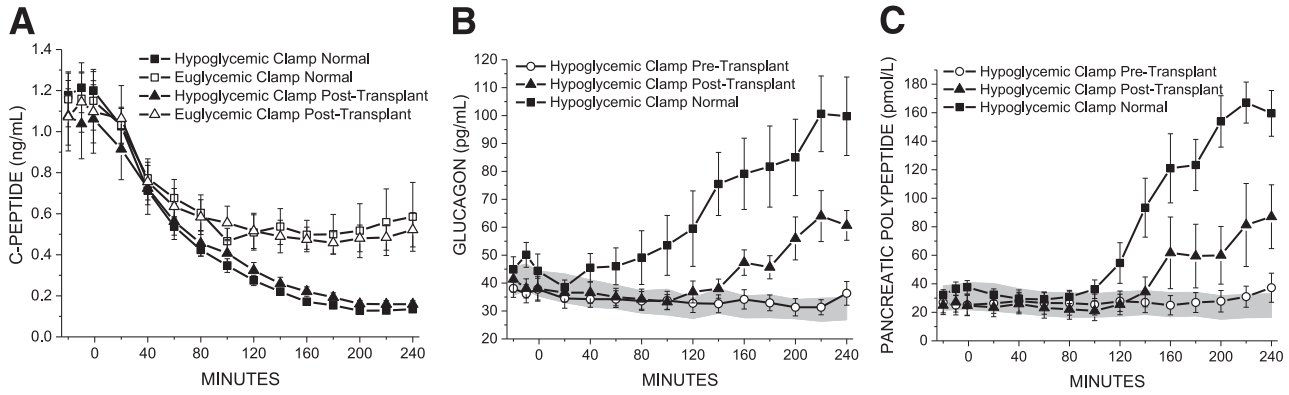

Figure 1—Islet cell hormonal responses during the hyperinsulinemic-hypoglycemic clamp in T1D subjects before (○) and 6 months after (▲) islet transplantation ($n = 12$) and in nondiabetic control subjects (■) ($n = 8$). For C-peptide (*A*), data are not shown before transplantation when undetectable (<0.1 ng/mL), and the response during the euglycemic clamps is shown for the T1D subjects 6 months after transplantation (△) ($n = 12$) and in the control group (□) ($n = 8$). For glucagon (*B*) and pancreatic polypeptide (*C*), the shaded area represents the 95% CI for data derived from all the hyperinsulinemic-euglycemic control experiments ($n = 32$).

Table 2—Magnitude[a] of C-peptide suppression and counterregulatory responses

	T1D subjects		
	Before islet transplantation	After islet transplantation	Nondiabetic control subjects
n	12	12	8[b]
C-peptide (ng/mL)	–	0.16 ± 0.02	0.13 ± 0.01
Glucagon (pg/mL)	33 ± 3	60 ± 7**	95 ± 13**†
PP (pmol/L)	32 ± 8	76 ± 23*	160 ± 16**†
Epinephrine (pg/mL)	130 ± 16	253 ± 21**	419 ± 46**‡
Autonomic symptoms (Δ)	2.2 ± 0.9	5.3 ± 1.0§	6.9 ± 2.4*
EGP (mg · kg^{-1} · min^{-1})	0.59 ± 0.12	1.18 ± 0.13*	1.42 ± 0.14**
Free fatty acids (μmol/L)	50 ± 7	161 ± 37*	114 ± 18**

Data are mean ± SE. PP, pancreatic polypeptide. [a]The magnitude of each hormonal, EGP, free fatty acid, and incremental symptom response to the hypoglycemic clamps was assessed as the mean of values obtained during the last 60 min of each clamp. [b]*n* = 6 for epinephrine. *$P < 0.05$ for comparison with T1D subjects before transplantation. **$P < 0.01$ for comparison with T1D subjects before transplantation. §$P = 0.06$ for comparison with T1D subjects before transplantation. †$P < 0.05$ for comparison with T1D subjects after islet transplantation. ‡$P < 0.01$ for comparison with T1D subjects after islet transplantation.

during hypoglycemia in the T1D subjects after transplantation ($P < 0.01$), albeit to levels that remained less than in control subjects ($P < 0.05$) (Fig. 1*B* and Table 2). Pancreatic polypeptide failed to activate in the T1D subjects before transplantation but did respond to hypoglycemia after transplantation ($P < 0.05$), although again to levels less than in control subjects ($P < 0.05$) (Fig. 1*C* and Table 2).

Sympathoadrenal Responses

Epinephrine secretion was markedly impaired during hypoglycemia in the T1D subjects before transplantation ($P < 0.01$ vs. control subjects) and improved after transplantation ($P < 0.01$), but the magnitude of the response remained less than in control subjects ($P < 0.01$) (Fig. 2*A* and Table 2). Autonomic symptoms were also diminished during hypoglycemia in the T1D subjects before transplantation ($P < 0.05$ vs. control subjects), with a trend toward improved symptom responses after transplantation ($P = 0.06$) that were not different from that in control subjects (Fig. 2*B* and Table 2).

EGP and Free Fatty Acids

EGP was suppressed during hypoglycemia in the T1D subjects before transplantation ($P < 0.01$ vs. control subjects) to levels not different from under euglycemic conditions, whereas EGP increased in response to hypoglycemia after transplantation ($P < 0.05$) such that the magnitude of the response was not different from that in control subjects (Fig. 2*C* and Table 2). Free fatty acids were suppressed during hypoglycemia in the T1D subjects before transplantation ($P < 0.01$ vs. control subjects) but increased in response to hypoglycemia after transplantation ($P < 0.05$) such that the magnitude of the response was not different from that in control subjects (Table 2).

DISCUSSION

The current study demonstrates that intrahepatic islet transplantation can restore glucose counterregulation and improve hypoglycemia symptom recognition in patients with long-standing T1D. To our knowledge, these are the first data to show that the EGP response to insulin-induced

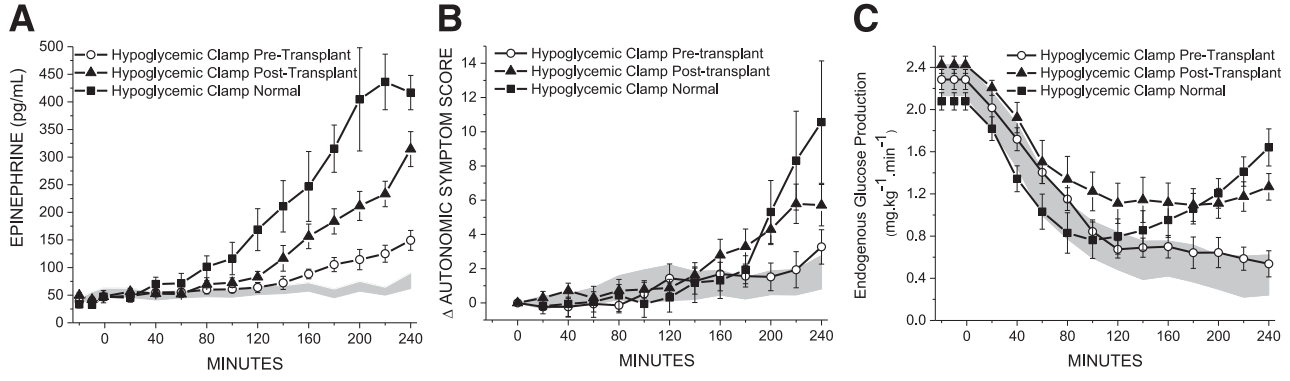

Figure 2—Sympathoadrenal (epinephrine [*A*] and autonomic symptoms [*B*]) and EGP (*C*) responses during the hyperinsulinemic-hypoglycemic clamp in T1D subjects before (○) and 6 months after (▲) islet transplantation ($n = 12$) and in nondiabetic control subjects (■) ($n = 8$, except for epinephrine, where $n = 6$). The shaded area represents the 95% CI for data derived from the hyperinsulinemic-euglycemic control experiments ($n = 32$, except for epinephrine, where $n = 30$).

hypoglycemia, absent in the patients with long-standing T1D before transplantation, was normalized by 6 months after transplantation. Because the ability to increase EGP is ultimately required to circumvent the development of low blood glucose, this finding helps to explain the dramatic effect of clinical islet transplantation on the amelioration of problematic hypoglycemia in T1D patients with severe hypoglycemia unawareness.

These results extend by longitudinal design to prior cross-sectional studies indicating that intrahepatic islets respond appropriately to insulin-induced hypoglycemia by suppressing endogenous insulin secretion (6,7) and partially restoring glucagon (7) and epinephrine secretion (8). That the magnitude of the glucagon response is less than normal may be explained by the mass of surviving islets being less than normal as estimated by measurement of the β-cell secretory capacity. In fact, the glucagon response after islet transplantation reported here appears greater than in our prior studies (7,8) and is consistent with the significant improvement in β-cell secretory capacity to ~40–50% of normal that we reported using the CIT07 protocol for islet transplantation (9). Although epinephrine can augment glucagon secretion (15), it stands to reason that the glucagon response is from transplanted rather than from native islets because patients with long-standing T1D who have normal epinephrine responses to insulin-induced hypoglycemia still fail to release glucagon (16). An alternative explanation for the partial glucagon response posits inhibition of intrahepatic α-cells by the resulting increased EGP because local glucose levels may be higher than that measured peripherally (17). However, the normal suppression of C-peptide during hypoglycemia indicates that intrahepatic β-cells appropriately sense and respond to the degree of peripheral hypoglycemia. Whether alternative sites for islet transplantation allow for greater glucagon responses (18) and glucose counterregulation requires further study.

Abolition of hypoglycemia after islet transplantation was documented by CGMS, indicating almost no time spent <70 mg/dL. This finding is consistent with prior reports on glycemic control evaluated by CGMS in islet recipients, including a large number of subjects using insulin to maintain near normoglycemia (19–21). The avoidance of time spent hypoglycemic would be expected to ameliorate HAAF. Indeed, the markedly impaired epinephrine secretion before transplantation was significantly improved after transplantation, and autonomic symptoms, also impaired before transplantation, improved by trend and were not different from control subjects after transplantation. Pancreatic polypeptide secretion, a marker of parasympathetic (vagal) activation of the islet that failed to activate before transplantation, responded to hypoglycemia after islet transplantation and likely represents another marker for reversal of HAAF (16).

That the partially normal glucagon and epinephrine responses were associated with complete normalization of the EGP response to insulin-induced hypoglycemia may be explained by the change in glucagon level, rather than its absolute concentration, being most important for stimulating EGP in humans (22) and that glucagon and epinephrine operate synergistically to increase EGP (23). The increase in free fatty acids during insulin-induced hypoglycemia after, but not before, transplantation suggests correction of defective activation of lipolysis best explained by the improved epinephrine levels (24). Free fatty acids are an important fuel substrate for glucose production by the liver and, together with the effects of improved glucagon and epinephrine levels on the liver, likely contributed to restoration of the EGP response. The present results are consistent with those of a recent cross-sectional study that included intrahepatic islet transplant recipients with partial graft function associated with partial restoration of the EGP response to insulin-induced hypoglycemia (25), suggesting that a threshold for counterregulatory hormone responses may exist for normalization of EGP.

In conclusion, intrahepatic islet transplantation can restore glucose counterregulation and improve hypoglycemia symptom recognition in patients with long-standing T1D. Although the adverse effects of the required immunosuppression preclude broad application in T1D, and further work should establish the durability of improved glucose counterregulation, islet transplantation should be considered as a treatment option for patients with severely problematic hypoglycemia.

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