Long-term improvement in glucose control and counterregulation by islet transplantation for type 1 diabetes

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Context: Islet transplantation has been shown to improve glucose counterregulation and hypoglycemia symptom recognition in patients with type 1 diabetes (T1D) complicated by severe hypoglycemia episodes and symptom unawareness, but long-term data are lacking.

Objective: To assess the long-term durability of glucose counterregulation and hypoglycemia symptom responses 18 months after intrahepatic islet transplantation, and associated measures of glycemic control during a 24 month follow-up period.

Design, Setting, and Participants: Ten patients with T1D disease duration ≥27 years were studied longitudinally before and 6 and 18 months post-transplant in the Clinical & Translational Research Center of the University of Pennsylvania, and compared to 10 nondiabetic control subjects.

Intervention: All 10 patients underwent intrahepatic islet transplantation according to the CIT07 protocol at the Hospital of the University of Pennsylvania.

Main Outcome Measures: Counterregulatory hormone, endogenous glucose production, and autonomic symptom responses derived from stepped hyperinsulinemic-hypoglycemic and paired hyperinsulinemic-euglycemic clamps with infusion of 6,6-²H₂-glucose.

Results: Near-normal glycemia (HbA₁c ≤6.5%; time 70 – 180 mg/dl ≥95%) was maintained for 24 months in all patients with one returning to low-dose insulin therapy. In response to insulin-induced hypoglycemia, glucagon secretion was incompletely restored at 6 and 18 months, epinephrine was improved at 6 and normalized at 18 months, and endogenous glucose production and symptoms, absent before, were normalized at 6 and 18 months post-transplant.

Conclusions: In patients with T1D experiencing problematic hypoglycemia, intrahepatic islet transplantation can lead to long-term improvement of glucose counterregulation and hypoglycemia symptom recognition, physiologic effects that likely contribute to glycemic stability post-transplant.

Hypoglycemia is a major barrier to the achievement of adequate glycemic control for most patients with type 1 diabetes (T1D) (1). The American Diabetes Association treatment guidelines recommend that adults with T1D target HbA₁c levels < 7.0% unless there is a reason, such as significant hypoglycemia or hypoglycemia unawareness, to set a higher target < 8.0% (2). However, even at a HbA₁c < 7.0% the residual risk for cardiovascular and all-cause mortality in T1D patients remains more than twice that in nondiabetic control subjects (3), with the lowest mortality rates seen with HbA₁c ≤ 6.5% (4), supporting advocacy of a lower target when possible.
without problematic hypoglycemia (5). Unfortunately, despite tremendous advances in the technology available for insulin delivery and glucose monitoring, only 30% of adults with T1D in the United States receiving care at specialty diabetes clinics are achieving HbA1c levels < 7.0% (6). Moreover, 8% reported experiencing severe hypoglycemia resulting in seizure or loss-of-consciousness in the prior 3 months, including 6% of those with HbA1c levels < 7.0% (6). Thus, current data do not support the historical link between HbA1c levels and severe hypoglycemia risk, and so recommendations to set higher HbA1c targets are unlikely to impact the burden of severe hypoglycemia in T1D. Moreover, the targeting of higher HbA1c levels is often not acceptable to patients striving to avoid or mitigate the development of microand macrovascular complications. Clearly, there is a need for further improvement in the approaches to diabetes management in order to realize the benefits of near-normal glycemic control without the accompanying risk for severe hypoglycemia faced by every person with insulin-dependent diabetes.

The risk of experiencing a severe hypoglycemic episode increases with the duration of disease, being 3 times greater with more than 15 years compared to less than 5 years of disease duration (7). This increased risk with longer disease duration is related to progressive development of compromised physiologic defense mechanisms against a falling plasma glucose concentration in the setting of therapeutic hyperinsulinemia. By 15 years of disease duration most patients with T1D have developed near-total loss of functioning β-cells (C-peptide negative) (8) resulting in loss of inhibition of endogenous insulin secretion as well as activation of glucagon secretion in response to declining blood glucose (9), which together normally increase endogenous (primarily hepatic) glucose production (EGP) to circumvent the development of hypoglycemia. In the absence of these islet cell responses to hypoglycemia, epinephrine secretion and autonomic symptom generation become critical to increase EGP and alert individuals to ingest food (10). Unfortunately, these sympathoadrenal responses are impaired by recurrent episodes of hypoglycemia leading to a syndrome of hypoglycemia unawareness, also known as hypoglycemia-associated autonomic failure (HAAF) (11). The presence of impaired awareness of hypoglycemia in T1D is associated with a six-fold increased risk for experiencing severe hypoglycemia (12, 13), that may be as high as twenty-fold with unawareness (12). The occurrence of severe hypoglycemia events contributes significantly to diabetes morbidity (6) and mortality (14).

Importantly, not every patient with reduced awareness of hypoglycemia experiences severe hypoglycemia episodes, and additional risk is imparted by glucose variabil-

Subjects and Methods

Subjects included in this study had long-standing C-peptide negative T1D that, despite participation in intensive diabetes management, was complicated by hypoglycemia unawareness (Clarke score ≥ 4) and at least one episode of severe hypoglycemia in the prior year associated with a hypoglycemic severity (HYPO) score ≥ 1047 and/or a glycemic lability index (LI) ≥ 433 mmol/L²/h·wk⁻¹, and who underwent islet transplantation as part of the CIT07 protocol at the University of Pennsylvania (22), and were followed for two-years (n = 10). One subject who completed the previously reported 6 month glucose counterregulatory testing (19) was unable to complete subsequent CGM or counterregulatory assessments, and so was excluded from this analysis although remained insulin-independent after two islet infusions with HbA1c 5.7% at two-years post-transplant. The T1D subjects underwent one or two intraportal infusions of islets in order to achieve insulin-independence. Maintenance immunosuppression consisted of low-dose tacrolimus (12-hour blood
through target 3 – 6 µg/L) and sirolimus (24-hour blood trough target 10 – 15 µg/L for the first 3 months and 8 – 12 µg/L thereafter). Two-year outcomes for the entire CIT07 cohort (n = 48) has recently been reported (23).

Healthy nondiabetic control subjects (n = 10) were sex-, age-, and BMI-matched to the T1D subjects. The study protocols were approved by the Institutional Review Board of the University of Pennsylvania, and all subjects gave their written informed consent to participate.

Continuous glucose monitoring

T1D subjects were evaluated using a 72-hour continuous glucose monitoring system (CGMS; Medtronic Minimed, Northridge, CA) prior to and at 75 days, 12 months and 24 months following islet transplantation. All subjects utilized a study glucometer (OneTouch Ultra, LifeScan, Milpitas, CA) to calibrate the CGMS 4 times daily with no interval between readings exceeding 12 hours.

Assessment of glucose counterregulation

T1D subjects underwent paired hyperinsulinemic-hypoglycemic and euglycemic clamps prior to and at 6 and 18 months following their final islet infusion. One subject received a second islet infusion after completing the 6 month assessment, and was studied again at 18 months after this final transplant. Each pair of clamps was conducted with at least one week and not more than one month between studies with the order of hypoglycemic vs. euglycemic condition determined by block randomization. T1D subjects were admitted to the Clinical and Translational Research Center the afternoon before study and fasted overnight after 2000 for 12 hours before testing. T1D subjects prior to transplantation were converted from subcutaneous insulin to a low-dose intravenous (IV) insulin infusion at 2100 the evening before study to target blood glucose at 81 – 115 mg/dL overnight. By 700, one catheter was placed in an antecubital vein for infusions, and one catheter was placed in a hand or forearm vein for blood sampling, with the hand or forearm placed in a thermostatted box (~50°C) or heating pad to promote arterialization of venous blood.

At t = 120 minutes a primed (5 mg/kg-fasting plasma glucose in mg/dL/90 for 5 minutes) continuous (0.05 mg/kg • min⁻¹ for 355 minutes) infusion of the stable glucose isotope tracer 6,6-2H₂-glucose (99% enriched; Cambridge Isotopes Laboratories, Andover, MA) was administered to assess EGP before and during the induction of hyperinsulinemia (24). When used overnight, the insulin infusion was continued during this period to maintain stable normoglycemia until t = 0. After baseline blood sampling at ~20, ~10, and ~1 minute, at t = 0 minutes a continuous infusion of insulin was initiated at 1 mU/kg • min⁻¹ for 240-minute to produce hyperinsulinemia. Subsequently, a variable rate infusion of 20% glucose was administered according to the glycemic clamp technique to achieve hourly plasma glucose steps of ~80, 65, 55, and 45 mg/dL. To reduce changes in plasma enrichment of 6,6-2H₂-glucose during the clamp, the 20% glucose solution was enriched to ~2.0% with 6,6-2H₂-glucose (24). Plasma glucose was measured every 5 minutes at bedside with an automated glucose analyzer (YSI 2300; Yellow Springs Instruments, Yellow Springs, OH) to adjust the glucose infusion rate and achieve the desired plasma glucose concentration. Additional blood samples for biochemical analysis and an autonomic symptom questionnaire (20) were collected every 20 minutes.

The hyperinsulinemic-euglycemic clamp was conducted as described for the hypoglycemic clamp above but with the target plasma glucose ~90 mg/dl for the entire 240-minute study.

All samples were collected on ice into chilled tubes containing EDTA, with Protease Inhibitor Cocktail (Sigma-Aldrich, St. Louis, MO) added to the tubes for peptide hormones, centrifuged at 4°C, separated, and frozen at ~−80°C for subsequent analysis. Plasma glucose was verified in duplicate by the glucose oxidase method using an automated glucose analyzer (YSI 2300). Plasma insulin, glucagon, and pancreatic polypeptide were measured in duplicate by double-antibody radioimmunoassays (Millipore, Billerica, MA for insulin and glucagon; ALPICO Diagnostics, Windham, NH for pancreatic polypeptide). Plasma epinephrine was measured by high-performance liquid chromatography (HPLC) with electrochemical detection. Enrichment of 6,6-2H₂-glucose was measured by gas chromatography-mass spectrometry. Plasma free fatty acids levels were measured in duplicate using enzymatic colorimetrics (Wako Chemicals, Richmond, VA).

Calculations and statistics

Measures of hypoglycemia unawareness (Clarke score), hypoglycemia severity (HYPO score), and the glycemic lability index (LI) were calculated from questionnaires, event diaries, and self-monitoring of blood glucose records, respectively, as previously described (18). CGMS measures required > 48 hours of valid interstitial glucose recording for calculation of glucose mean, SD, CV (25), proportion of time spent with hyperglycemia (>180 mg/dL), and proportion of time spent with varying degrees of hypoglycemia (<70 mg/dL, <60 mg/dL, and <54 mg/dL) (7, 26). The rate of appearance (Rₜₐₜ) of glucose during the clamps was calculated using Steele’s nonsteady state equation modified for the use of stable isotopes (27). 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, incremental symptom, EGP, and free fatty acid response was assessed as the mean of values obtained during the last 60 minutes of hypoglycemia.

All data are expressed as mean ± SE unless otherwise noted. Comparison of results within the T1D subjects from preto post-transplant times of assessment was performed by Friedman ANOVA, and when significant, pairwise comparisons were performed using the Wilcoxon-matched pairs test, while comparison of results between each T1D time of assessment and nondiabetic controls was performed with the Mann-Whitney U test using Statistica software (StatSoft, Inc., Tulsa, OK). Significance was considered at P < .05 (two-tailed).

Results

Subject characteristics

The T1D subjects (n = 10) had a disease duration of 27 ± 4 years, and were of comparable gender distribution, age, and BMI with the nondiabetic control subjects (n = 10; Table 1). HbA₁c, which was elevated before, decreased to nondiabetic levels after islet transplantation and remained ≤ 6.5% in all subjects during the 24 month follow-up period (P < .001; Figure 1A). Insulin require-
glucose counterregulation and islet transplants J Clin Endocrinol Metab

Table 1. Subject Characteristics

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<th>Sex (M/F)</th>
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<td>M</td>
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T1D

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<td>Control 9</td>
<td>F</td>
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<td>Control 10</td>
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Non-diabetic

<table>
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<td>4/6</td>
<td>46 ± 2</td>
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Data are means ± se. T1D, type 1 diabetes at assessment before islet transplantation.

ments were ~0.5 U·kg⁻¹·d⁻¹ before transplantation, and were eliminated in all but one subject who returned to requiring ~0.1 U·kg⁻¹·d⁻¹ after 12 months to maintain near-normal glycemic control (P < .001; Figure 1B). Seven subjects were insulin-independent following one islet infusion, and three were insulin-independent following two islet infusions. The total islet equivalents/kg transplanted per recipient was 9845 ± 685. Subjects maintained appropriate levels of tacrolimus and sirolimus except for two who were unable to tolerate sirolimus and converted to mycophenolate mofetil.

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 (Figure 1C), HYPO score 2769 ± 847, and LI (Figure 1D), respectively, before transplantation. Following transplantation, no subject experienced any clinically significant hypoglycemia, the Clarke score became and remained negligible through 24 months (P < .001; Figure 1C), the HYPO score became 0 in all subjects when reassessed at 12 months (P < .01), and LI was markedly reduced at 6 months, becoming negligible by 12 months (P < .01; Figure 1D).

Continuous glucose monitoring

CGM demonstrated sustained improvement in mean glucose through 24 months post-transplant (P = .01; Table 2), substantial reduction in glycemic variability assessed as either glucose SD or CV (P = .001 for both; Table 2), and almost no time spent hyperglycemic (glucose > 180 mg/dL) or hypoglycemic (glucose < 70 mg/dL) (P < .001 for all comparisons; Table 2), such that during the periods of assessment over 24 months ≥ 95% of time was spent in the target range of 70 – 180 mg/dL (2, 26).

Counterregulatory responses during the hypoglycemic clamp

The insulin infusion administered during the hypoglycemic clamp resulted in comparable hyperinsulinemia in the T1D subjects preand at 6 and 18 months post-transplant, and in the nondiabetic controls, which was also not different in any group from the hyperinsulinemia achieved during their respective euglycemic control experiments (Figure 2A). During the hypoglycemic clamp, plasma glucose at the 80 mg/dL step was higher by trend prewhen compared to 6 and 18 months post-transplant (92 ± 4 vs. 82 ± 2 vs. 82 ± 1 mg/dL; P = .07) and when compared to control (81 ± 2 mg/dL; P < .05), and thereafter was comparable at the 65 (66 ± 2 vs. 63 ± 2 vs. 66 ± 1 vs. 65 ± 1), 55 (55 ± 2 vs. 52 ± 2 vs. 55 ± 1 vs. 57 ± 1), and 45 (46 ± 1 vs. 45 ± 1 vs. 46 ± 1 vs. 49 ± 1) mg/dL hourly steps, whereas during the euglycemic clamp, plasma glucose remained between 85 – 90 mg/dL (Figure 2B). Plasma enrichment of 6,6-2H₂-glucose increased modestly from baseline to 100 minutes and was stable thereafter, and is given together with the glucose Ra and glucose infusion rates during the clamps in the Supplementary Figure.

Glucagon failed to activate during the hypoglycemic clamp in the T1D subjects pretransplant (P<0.01 vs. controls), with levels not different than under euglycemic conditions, whereas glucagon increased during the hypoglycemic clamp in the T1D subjects at 6 and 18 months post-transplant (P < .01 each vs. pretransplant), albeit incompletely to levels less than in controls (P < .05 for both 6 and 18 months vs. controls; Figure 3A; Table 3). The pancreatic polypeptide response was substantially impaired in the T1D subjects pretransplant (P<0.01 vs. controls), and improved at 6 and 18 months post-transplant (P ≤ .01 each vs. pretransplant), although again to levels less than in controls (P < .05 for 6 months and P = .06 for 18 months vs. controls; Figure 3B; Table 3).

Epinephrine secretion was markedly impaired during hypoglycemia in the T1D subjects pretransplant (P<0.01 vs. controls), and improved at 6 and 18 months post-transplant (P < .01 each vs. pretransplant), with the magnitude of the response still less than in controls at 6 months (P < .01), and increasing to levels not different than in controls at 18 months (P < .01 vs. 6 months; Figure 3C; Table 3). Autonomic symptoms were greatly diminished during hypoglycemia in the T1D subjects pretransplant (P<0.01 vs.
controls), with scores not different than under euglycemic conditions, with a trend for improved symptom responses at 6 months (P/H 0.1 vs. pretransplant) that was significant at 18 months (P/H < 0.01 vs. pretransplant) and not different at either time after transplantation from that in controls (Figure 3D; Table 3).

EGP was suppressed during the hypoglycemic clamp in the T1D subjects pretransplant (P/H < 0.01 vs. controls) to levels not different than under euglycemic conditions, whereas EGP increased in response to hypoglycemia at 6 and 18 months post-transplant (P < 0.05 each vs. pretransplant) such that the magnitude of the response was not different than in controls (Figure 3E; Table 3). Free fatty acid levels were suppressed during the hypoglycemic clamp in the T1D subjects pretransplant (P/H < 0.01 vs. controls), but increased in response to hypoglycemia at 6 and 18 months post-transplant (P ≤ 0.01 each vs. pretransplant).

### Table 2. Continuous Glucose Monitoring System (CGMS) Measures

<table>
<thead>
<tr>
<th>Time from transplant</th>
<th>pre</th>
<th>75 days post</th>
<th>12 months post</th>
<th>24 months post</th>
<th>P valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean glucose (mg/dl)</td>
<td>166 ± 13</td>
<td>118 ± 3</td>
<td>118 ± 5</td>
<td>112 ± 4</td>
<td>0.01</td>
</tr>
<tr>
<td>Glucose ≥ (mg/dl)</td>
<td>75 ± 6</td>
<td>20 ± 3</td>
<td>19 ± 2</td>
<td>21 ± 2</td>
<td>0.001</td>
</tr>
<tr>
<td>Glucose CV (%)</td>
<td>45 ± 2</td>
<td>17 ± 2</td>
<td>16 ± 1</td>
<td>19 ± 2</td>
<td>0.001</td>
</tr>
<tr>
<td>Time &gt;180 mg/dl (%)</td>
<td>39 ± 6</td>
<td>1 ± 1</td>
<td>3 ± 2</td>
<td>1 ± 0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time &lt;70 mg/dl (%)</td>
<td>13 ± 3</td>
<td>2 ± 1</td>
<td>1 ± 0</td>
<td>4 ± 2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time &lt;60 mg/dl (%)</td>
<td>9 ± 2</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>1 ± 1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time &lt;54 mg/dl (%)</td>
<td>6 ± 2</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>1 ± 1</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are means ± ss. Evaluable CGMS data was available for all (n = 10) but one subject at 75 days and one different subject at 24 months post-transplant. *P value for Friedman ANOVA comparison of results within the T1D subjects from pre- to post-transplant times of assessment.

Figure 1. Clinical measures of A, glycemic control (HbA1c), B, insulin requirements, C, hypoglycemia unawareness (Clarke score), and D, glycemic lability index (LI) in subjects with type 1 diabetes (n = 10) before and at various intervals after islet transplantation to 24 months. In A, normal data for HbA1c, are derived from the non-diabetic control subjects (n = 10). In B, post-transplant insulin use is accounted for by one subject who received a second islet infusion after 6 months, was insulin free at 12 months following this second islet infusion, and returned to insulin use thereafter. In C, a Clarke score of 4 or more indicates reduced awareness of hypoglycemia. In D, the LI requires records of 4 or more self-monitoring blood glucose values daily, which were only available for a subset of the cohort at 12 months post-transplant (n = 6). The box plots represent the median, upper and lower quartiles, mean (□), and range (error bars).
Discussion

These results indicate that intrahepatic islet transplantation can lead to long-term improvement in glucose counterregulation and hypoglycemia symptom recognition in T1D where these responses to insulin-induced hypoglycemia were absent prior to transplantation. While the partial glucagon response seen at 6 months remained incomplete at 18 months, the epinephrine response that was only partially improved at 6 months became fully normalized at 18 months, with a similar effect seen for pancreatic polypeptide secretion, a marker of parasympathetic (vagal) activation, and autonomic symptom generation. The additional improvement in the epinephrine response from 6 to 18 months is best explained by the longer duration without exposure to hypoglycemia as documented by CGM allowing for complete reversal of HAAF, and indicates that recovery of autonomic nervous system function in those most severely affected by HAAF requires similar avoidance of hypoglycemia for more than a 6 month period.

Glucose counterregulation is best defined by the increase in EGP that is ultimately required to circumvent the development of low blood glucose. Here, there is the appearance of a delay in the partial glucagon response to hypoglycemia. This finding is consistent with the hypothesis that intrahepatic glucose release may attenuate the exposure of intrahepatic islets to peripheral hypoglycemia (28). It is possible that release of glucagon from islets within the liver may create an early increase in EGP with subsequent attenuation in the stimulated glucagon levels measured peripherally, yet avoiding the development of low blood glucose. Unfortunately, the increasing plasma enrichment of glucose during the first 100 minutes reported here precludes making comparisons of EGP during this time. As previously reported (19) the incomplete glucagon together with a partial epinephrine response at 6 months following transplantation was associated with normal EGP and free fatty acid responses to insulin-induced hypoglycemia. And while only one of our subjects required low-dose insulin treatment post-transplant, a cross-sectional study of insulin-dependent islet transplant recipients demonstrated modest increments in glucagon and epinephrine with partial recovery of EGP seen over the suppressed rates observed in T1D subjects (29). Thus, improvement of glucose counterregulation is observed as well in islet transplant recipients with partial graft function that can explain the protection afforded by islet grafts against problematic hypoglycemia even when insulin is required to maintain near-normoglycemia.

Indeed, CGM studies have demonstrated similar reductions in mean glucose, glucose variability, and time spent hypoglycemic (<34 mg/dL) relative to T1D for both insulin-independent and insulin-requiring islet recipients (30, 31). That the islet graft is responsible for these improvements in CGM measures of glycemic control is supported by the demonstration of significant continuous associations with stimulated C-peptide levels in islet transplant recipients (32). Thus, while the substantially improved CGM measures of glycemic control reported here may be required over the long-term to fully reverse HAAF, even partial improvements in glucose counterregulation and hypoglycemia symptom recognition likely work in concert with reduced glucose variability to provide the robust protection from hypoglycemia afforded by this strategy in patients with T1D.
islet transplantation. The parent CIT07 study recently reported 93% probability for remaining free of severe hypoglycemia events for up to 2 years post-transplant (23).

Interestingly, similar protection from hypoglycemia as demonstrated here for T1D patients undergoing intrahepatic islet allo-transplantation has not been observed for chronic pancreatitis patients undergoing total pancreatectomy with islet auto-transplantation (TPIAT). CGM study of insulin-independent TPIAT recipients has demonstrated ~15% of time spent < 70 mg/dL (33), similar to that observed in our T1D subjects before transplantation and quite different from the < 4% of time documented over 24 months following islet allo-transplantation. As most hypoglycemic episodes reported in TPIAT patients occur postprandially, alimentary hypoglycemia attributable to the roux-en-Y gastrointestinal (GI) reconstruction seems the most plausible explanation, with the reported impairments in glucagon secretion and autonomic symptoms (33) reflecting the induction of HAAF, rather than its reversal as demonstrated here for islet allo-transplantation.

While our subjects all participated in intensive diabetes

Figure 3. Counterregulatory hormone (glucagon, A, pancreatic polypeptide, B, epinephrine, C), autonomic symptom, D, endogenous glucose production, E, and free fatty acid, D, responses during the hyperinsulinemic hypoglycemic clamp in type 1 diabetes subjects before (-■-) and at 6 months (-▲-) and 18 months (-❖-) following islet transplantation (n = 10), and in nondiabetic control subjects (-▼, n = 10). The shaded area represents the 95% CI for data derived from the hyperinsulinemic euglycemic control experiments (n = 40). The preand 6 month post-transplant data contributed to a prior report (ref. 19).
management prior to their selection for islet transplantation, many T1D patients experiencing problematic hypoglycemia are not receiving such care. Participation in structured education programs and optimization of insulin delivery and glucose monitoring including use of insulin pumps and real-time CGM as appropriate and if available can lead to a reduction in severe hypoglycemia events, improved hypoglycemia awareness, and decreased measures of hypoglycemia severity (34); however, none of these approaches have improved mean glucose or HbA1c in this population (35). An algorithm has been proposed for the implementation of educational and technologic interventions prior to consideration of isolated islet or whole pancreas transplantation to address problematic hypoglycemia (17). Evaluation of specific interventions in T1D patients with hypoglycemia unawareness have shown that real-time CGM with (36) or without (37) automated insulin suspension via communication to an insulin pump can lower rates of severe hypoglycemia, but without improving the epinephrine response to insulin-induced hypoglycemia or hypoglycemia awareness.

In conclusion, patients with long-standing T1D experiencing problematic hypoglycemia despite intensive diabetes management can achieve durable improvement in glucose counterregulation and hypoglycemia symptom recognition with intrahepatic islet transplantation. This is the first report in patients with >15 years disease duration and absent glucose counterregulation and hypoglycemia symptom recognition prior to intervention evidencing normalization of the epinephrine response to hypoglycemia. Remarkably, the recovery of glucose counterregulation with islet allo-transplantation is associated with near-normal glycemic control assessed over 2 years with almost no time spent hypoglycemic (<70 mg/dL). These results are distinct from those reported with TPIAT for chronic pancreatitis, and from outcomes seen with nontransplant interventions for T1D. Whole pancreas transplantation (38) remains the only alternative treatment for patients with T1D and problematic hypoglycemia that has been shown to achieve a composite endpoint of HbA1c <7.0% and elimination of severe hypoglycemia as reported for islet transplantation by several multicenter trials at 1 (39) and 2 (23, 40) years post-transplant. While presently the adverse effects of the required immunosuppression preclude broad application in T1D, islet transplantation should be considered as a treatment option for patients with problematic hypoglycemia failing nontransplant interventions.

Acknowledgments

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**Table 3.** Magnitude* of Counterregulatory Responses

<table>
<thead>
<tr>
<th>Time from transplant</th>
<th>T1D subjects, beforea</th>
<th>T1D subjects, 6 months afterb</th>
<th>T1D subjects, 18 months after</th>
<th>P valuec</th>
<th>Non-diabetic control subjects</th>
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<tbody>
<tr>
<td>Glucagon (pg/ml)</td>
<td>32 ± 3</td>
<td>61 ± 8**</td>
<td>61 ± 9**</td>
<td>&lt;0.01</td>
<td>93 ± 11**§</td>
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<td>PP (pmol/liter)</td>
<td>36 ± 9</td>
<td>86 ± 27**</td>
<td>107 ± 39**</td>
<td>&lt;0.01</td>
<td>149 ± 14**†‡</td>
</tr>
<tr>
<td>Epinephrine (pg/mL)</td>
<td>132 ± 19</td>
<td>269 ± 22**</td>
<td>483 ± 73**†</td>
<td>&lt;0.001</td>
<td>572 ± 71**†</td>
</tr>
<tr>
<td>Autonomic symptoms (Δ)</td>
<td>4.0 ± 1.2</td>
<td>6.6 ± 1.0*</td>
<td>8.9 ± 1.1**</td>
<td>&lt;0.05</td>
<td>10.4 ± 1.9**</td>
</tr>
<tr>
<td>EGP (mg·kg⁻¹·min⁻¹)</td>
<td>0.59 ± 0.14</td>
<td>1.14 ± 0.15</td>
<td>1.10 ± 0.13*</td>
<td>&lt;0.05</td>
<td>1.35 ± 0.12**</td>
</tr>
<tr>
<td>Free fatty acids (µmol/liter)</td>
<td>46 ± 7</td>
<td>174 ± 42**</td>
<td>172 ± 48**</td>
<td>&lt;0.01</td>
<td>112 ± 14**</td>
</tr>
</tbody>
</table>

Data are means ± se. PP, pancreatic polypeptide; EGP, endogenous glucose production. *The magnitude of each hormonal, incremental symptom, endogenous glucose production, and free fatty acid response to insulin-induced hypoglycemia was assessed as the mean of values obtained during the last 60 min of each hypoglycemic clamp. †The pre- and 6 month post-transplant data contributed to a prior report (ref. 19). §P value for Friedman ANOVA comparison of results within the T1D subjects from pre- to post-transplant times of assessment.

* P < 0.05 for comparison to T1D subjects before transplantation.

** P = 0.01 for comparison to T1D subjects before transplantation.

† P = 0.1 for comparison to T1D subjects before transplantation.

‡ P < 0.05 for comparison to T1D subjects 6 months after islet transplantation.

§ P < 0.01 for comparison to T1D subjects 6 months after islet transplantation.

¶ P < 0.05 for comparison to T1D subjects 18 months after islet transplantation.

£ P = 0.06 for comparison to T1D subjects 18 months after islet transplantation.
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