Intrahepatic Islet Transplantation in Type 1 Diabetic Patients Does Not Restore Hypoglycemic Hormonal Counterregulation or Symptom Recognition After Insulin Independence

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Islet allotransplantation can provide prolonged insulin independence in selected individuals with type 1 diabetes. Whether islet transplantation also restores hypoglycemic counterregulation is unclear. To determine if hypoglycemic counterregulation is restored by islet transplantation, we studied hormone responses and hypoglycemic symptom recognition in seven insulin-independent islet transplant recipients using a 3-h stepped hypoglycemic clamp, and compared their responses to those of nontransplanted type 1 diabetic subjects and nondiabetic control subjects. Glucagon responses of islet transplant recipients to hypoglycemia were significantly less than that observed in control subjects (incremental glucagon [mean ± SE]: −12 ± 12 vs. 64 ± 22 pg/ml, respectively; $P < 0.05$), and not significantly different from that of nontransplanted type 1 diabetic subjects (−17 ± 10 pg/ml). Epinephrine responses and symptom recognition were also not restored by islet transplantation (incremental epinephrine [mean ± SE]: 195 ± 128 [islet transplant recipients] vs. 238 ± 73 [type 1 diabetic subjects] vs. 633 ± 139 pg/ml [nondiabetic control subjects], $P < 0.05$ vs. control; peak symptom scores: 3.3 ± 0.9 [islet transplant recipients] vs. 3.1 ± 1.1 [type 1 diabetic subjects] vs. 6.7 ± 0.8 [nondiabetic control subjects]). Thus the results indicate that despite providing prolonged insulin independence and near-normal glycemic control in these patients with long-standing type 1 diabetes, hypoglycemic hormonal counterregulation and symptom recognition were not restored by intrahepatic islet transplantation. *Diabetes* 51:3428–3434, 2002

Intrahepatic islet transplantation has been demonstrated to restore insulin independence and improve glycemic control to near-normal in selected individuals with type 1 diabetes (1). The success of this procedure suggests that β-cells contained within transplanted islets secrete insulin appropriately in response to glycemic challenge. The degree to which α-cells, which are also contained within transplanted islets, respond to hypoglycemia (blood glucose <60 mg/dl) remains unclear. Normally, α-cells secrete glucagon in response to hypoglycemia, which then stimulates hepatic glucose production, thereby raising plasma glucose levels. Other hormones involved in counterregulation are epinephrine, norepinephrine, cortisol, and growth hormone. Among these hormones, glucagon and epinephrine are the most critical for rapid elevation of blood glucose during severe hypoglycemia (2).

Currently, the primary indications for islet transplantation are extreme glycemic lability and hypoglycemia unawareness, both of which put diabetic patients at risk of severe hypoglycemic events, including seizure and coma (1,3,4). Typically, patients with long-standing type 1 diabetes have severely impaired hormonal counterregulation (2,5). These patients are at especially high risk of severe hypoglycemia so that any therapy that improves counterregulatory responsiveness would be of great benefit to them.

Previously, whole organ pancreas transplantation was the only reliable means of restoring hypoglycemic counterregulation and normalizing glycemic control in patients with long-standing type 1 diabetes (6–10). Because transplanted islets contain α-cells, islet transplantation was also thought to have the potential to restore hormonal counterregulation. Until recently, this procedure was not successful enough to reliably determine whether restoration of α-cell responsiveness to hypoglycemia occurred. Improvements in the success of intrahepatic islet transplantation now provide the opportunity to examine counterregulation in this setting.

In the current study, we examined hypoglycemic hormonal counterregulation in islet allograft recipients with sustained insulin independence. The aim of the study was to determine whether glucagon and epinephrine responses and hypoglycemic symptom recognition are improved after successful islet transplantation. The results of the study provide insights into the physiology of α-cell function in the setting of intrahepatic islet transplantation. The results also may have implications for diabetic patients and their physicians who pursue islet transplantation as a reliable means of treating recurrent, severe hypoglycemia, and especially for those islet transplant recipients who continue to require exogenous insulin.

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TABLE 1
Subject characteristics

	Islet transplant recipients	Type 1 diabetic subjects	Nondiabetic control subjects
n	7	7	7
Sex (M/F)	6/1	4/3	3/4
BMI (kg/m^2)	23.0 ± 1.2	24.3 ± 1.4	24.2 ± 0.8
Age (years)	43 ± 3	39 ± 3	37 ± 5
Duration of diabetes (years)	27 ± 6	26 ± 3	—
Total islets transplanted per patient	840,155 ± 52,943	—	—
HbA_{1c} (%)	5.8 ± 0.1	9.7 ± 0.6	5.4 ± 0.1

Data are means ± SE.

RESEARCH DESIGN AND METHODS

Subjects. Seven islet allotransplant recipients, ages 30–54 years (mean age 43 ± 3 years) were examined at the University of Alberta Hospital between April and June 2001. The mean duration of insulin independence for all transplant recipients was 12.6 ± 0.6 months from the time of the final islet infusion. All recipients received transplants using the Edmonton Protocol for islet-alone transplantation (1). The primary indications for islet transplantation were the presence of type 1 diabetes for at least 5 years and either significant hypoglycemia unawareness, defined by the absence of adequate autonomic symptoms at plasma glucose levels <54 mg/dl (3.0 mmol/l), or severe metabolic instability, characterized by two or more episodes of severe hypoglycemia that required assistance from others over the 12 months before transplantation. Chronic immunosuppression in all patients consisted of combined low-dose sirolimus (rapamycin) and tacrolimus (FK-506) therapy in the absence of glucocorticoids. No subjects were taking glucose-lowering agents or β-blockers or had a history of epilepsy or active ischemic heart disease during the study. Results of the islet transplant group were compared to those of type 1 diabetic subjects and nondiabetic control subjects studied with the same stepped, hypoglycemic clamp technique. Four of the control subjects were studied in Edmonton at the same time as the islet transplant patients. The other three control subjects had been studied previously along with the type 1 diabetic subjects at the University of Minnesota. Each assay that included samples from an Edmonton control subject also contained samples from an Edmonton islet recipient.

The type 1 diabetic subjects and nondiabetic control subjects were matched for age and weight with the islet transplant subjects (Table 1). Fasting plasma glucose levels were normalized in all diabetic subjects using an overnight insulin infusion. All subjects gave informed, written consent before participation. The study was reviewed and approved by the Health Research Ethics Board of the University of Alberta.

Methods. All subjects underwent a 180-min stepped hypoglycemic clamp, as previously described (10). After a 12-h fast, intravenous access was obtained in a forearm vein of each subject for infusion of insulin and glucose. Venous blood was sampled from a separate intravenous catheter placed in the opposite arm. A blood sample was obtained every 5 min for the immediate determination of plasma glucose. After a 30-min equilibrium period, recombinant regular human insulin (Humulin; Lilly, Indianapolis, IN) was infused at 2 $mU \cdot kg^{-1} \cdot min^{-1}$. A variable-rate 20% glucose infusion was used to maintain blood glucose concentrations at glucose targets of 70, 60, 50, and 40 mg/dl at sequential 45-min intervals. Blood samples were obtained during the basal period and at 15-min intervals for the determination of glucagon and epinephrine concentrations. Heart rate and blood pressure were monitored throughout the study. Hypoglycemic symptoms were assessed every 45 min using the symptom score of Hoeldtke et al. (11).

A 30-min arginine stimulation test was performed separately from the hypoglycemic clamp studies on four of the seven islet transplant recipients. After an overnight fast, intravenous access was obtained in a forearm vein of each subject and normal saline was infused at a rate of 25 ml/h to keep the vein open. After a basal blood sample was drawn, 5 g of arginine, diluted to 40 ml with normal saline, were injected over 30 s, followed by a 20-ml saline flush. Blood samples were then drawn at 2, 3, 4, 5, 7, 10, 15, 20, 25, and 30 min after arginine injection for determination of glucagon concentrations.

Assays. Plasma glucose was measured at the bedside using an automated glucose analyzer (YSI Life Sciences, Yellow Springs, OH). Blood samples were collected in test tubes containing EDTA and aprotinin (500 units/ml) (Trasylol; Bayer) for glucagon and EGTA (90 mg/ml) and glutathione (60 mg/ml) for catecholamines. Samples were cooled on ice and then rapidly centrifuged. Serum was separated and stored at –70° C for subsequent analysis. Glucagon levels were determined by radioimmunoassay using the method described by Harris et al. (12). Epinephrine and C-peptide levels were determined using single-isotope radioimmunoassay techniques (13,14). All assays were performed by the same technician using identical techniques in all islet transplant subjects, type 1 diabetic patients, and healthy control subjects.

Statistical analysis. All statistical comparisons were performed using one-way ANOVA using the Sigmastat program. Results are expressed as means ± SE. Statistical significance was set at $P < 0.05$.

RESULTS

Glucose. The mean fasting plasma glucose levels of the islet transplant and control groups were 104 ± 0.2 and 90 ± 0.1 mg/dl, respectively (Fig. 1). The mean fasting plasma glucose level of the type 1 diabetic subjects after an overnight insulin infusion was 98 ± 0.9 mg/dl. There were no significant differences in the mean fasting and sequential 45-min glucose values among the three groups.

C-peptide. There were no statistical differences in the plasma C-peptide levels between the islet transplant recipients and control subjects during the clamp (Fig. 2). Type 1 diabetic subjects had undetectable C-peptide levels before the study (data not shown); therefore levels were not examined during the clamp.

Glucagon. The mean basal plasma glucagon level of the islet transplant group during the hypoglycemic clamp was higher than that seen in the type 1 diabetic subjects (210 ± 20 vs. 95 ± 19 pg/ml; $P < 0.05$), but was not significantly different from that of the control group (144 ± 23 pg/ml). A significant rise of plasma glucagon was observed in the

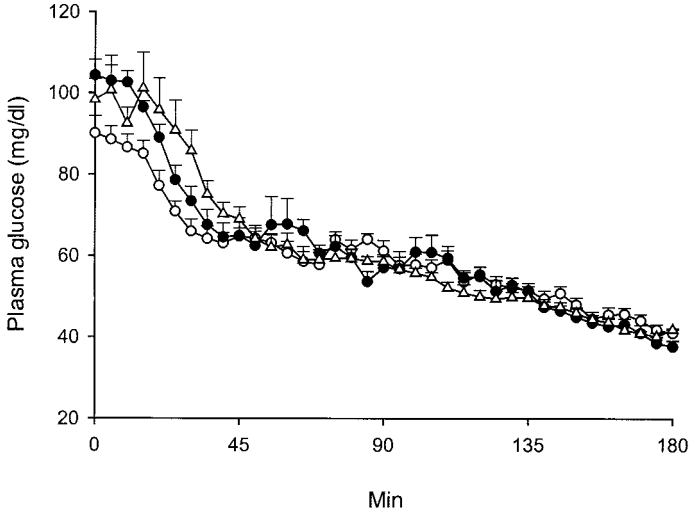


FIG. 1. Mean plasma glucose levels of islet transplant recipients (●), type 1 diabetic subjects (△), and nondiabetic control subjects (○) during the hypoglycemic clamp. Basal and sequential 45-min glucose levels were not significantly different among the three groups.

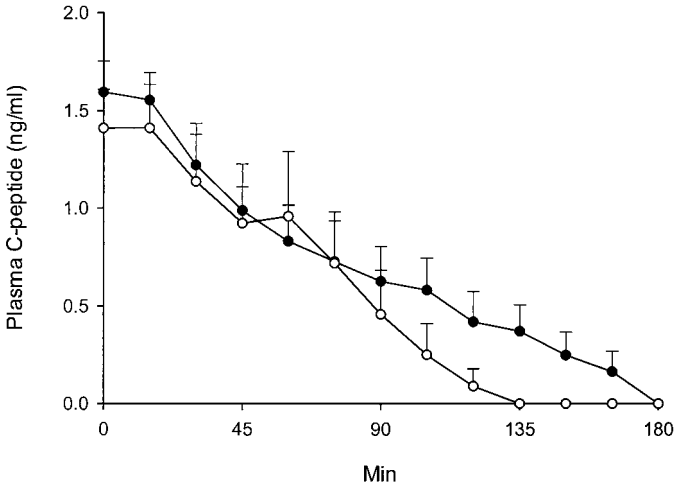
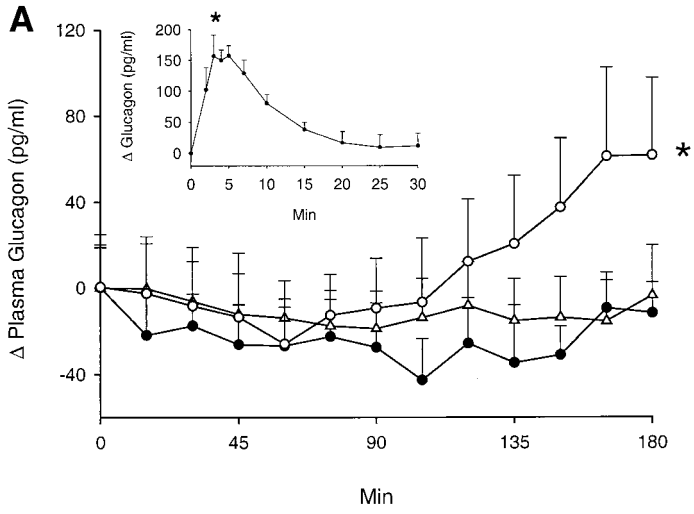


FIG. 2. Plasma C-peptide levels of islet transplant recipients (●) and nondiabetic control subjects (○) during the hypoglycemic clamp. There were no significant differences in C-peptide levels between the two groups throughout the clamp.



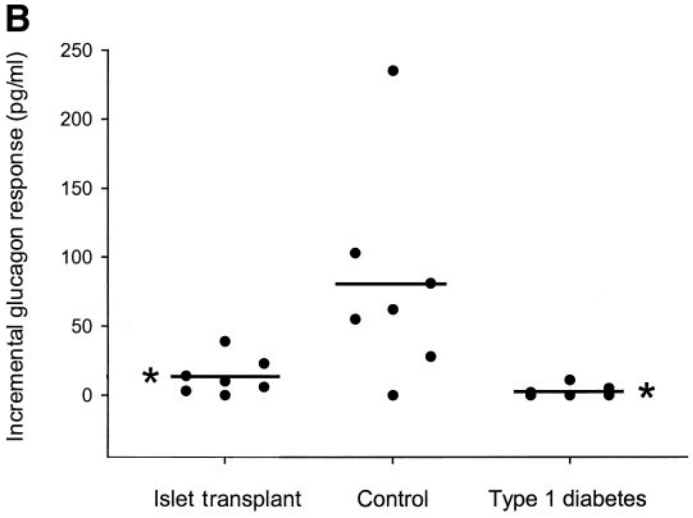


FIG. 3. *A*: Plasma glucagon response during hypoglycemic clamp of islet transplant recipients (●), type 1 diabetic subjects (△), and nondiabetic control subjects (○). A significant rise in plasma glucagon was observed in the control group. No significant rise in mean plasma glucagon was observed in the islet transplant recipients or diabetic subjects. *$P < 0.001$ vs. basal, ANOVA. *Inset*: Islet transplant recipients showed a significant glucagon response to intravenous arginine. *$P < 0.001$ vs. basal, ANOVA; $n = 4$. *B*: Incremental glucagon responses to hypoglycemia from baseline to 180 min of the islet transplant recipients (●), type 1 diabetic subjects (△), and nondiabetic control subjects (○). The mean incremental responses of the islet transplant recipients and the type 1 diabetic subjects did not differ significantly, but both were significantly less than that of nondiabetic control subjects. *$P < 0.05$ vs. control, ANOVA.

control group during the clamp (180-min glucagon: 208 ± 36 pg/ml; $P < 0.001$ vs. basal). No significant rise in the mean plasma glucagon level was observed in the islet transplant group or the type 1 diabetic group during the clamp (180-min glucagon: 198 ± 14 and 91 ± 22 pg/ml, respectively) (Fig. 3*A*). The mean incremental glucagon response (basal to 180 min) of the islet transplant group was significantly less than that of the control group (−12 ± 12 vs. 64 ± 22 pg/ml; $P < 0.05$) and was not significantly different from that of the type 1 diabetic group (−17 ± 10 pg/ml) (Fig. 3*B*). An intact glucagon response to arginine challenge (basal to peak) was observed in the four islet transplant recipients studied (158 ± 34 pg/ml; $P < 0.001$) (Fig. 3*A*, inset).

Epinephrine. The mean basal plasma epinephrine level of the islet transplant group was similar to that of the control group (18 ± 3 vs. 22 ± 3 pg/ml), but both were significantly less than that of subjects with long-standing type 1 diabetes (64 ± 5 pg/ml; $P < 0.05$). A significant rise in plasma epinephrine was observed in the control group during the clamp (180-min epinephrine: 655 ± 139 pg/ml; $P < 0.001$ vs. basal) (Fig. 4*A*). Two of the islet transplant recipients displayed an elevation of plasma epinephrine during the final 45 min of the clamp, but overall no significant rise in plasma epinephrine was observed in the islet transplant group. The mean incremental epinephrine response (basal to 180 min) of the islet transplant group was significantly less than that seen in the control group (195 ± 128 vs. 633 ± 139 pg/ml; $P < 0.05$), and not significantly different from that of subjects with long-standing type 1 diabetes (238 ± 73 pg/ml) (Fig. 4*B*).

Hypoglycemic symptom recognition. The mean symptom scores of the islet transplant recipients did not differ significantly from those of subjects with long-standing type 1 diabetes at all glycemic levels (Fig. 5). None of the islet transplant recipients recognized any symptoms of hypoglycemia until they had reached a plasma glucose level ≤50 mg/dl. At a glucose level of 40 mg/dl, the mean symptom scores of the islet transplant recipients and type 1 diabetic subjects were significantly less than those observed in nondiabetic control subjects (symptom scores: 3.3 ± 0.9, 3.1 ± 1.1, and 6.7 ± 0.7, respectively; $P < 0.05$ vs. control).

DISCUSSION

The results of the current study indicate that despite prolonged stable insulin independence and near-normal glycemic control, intrahepatic islet allotransplantation did not restore hypoglycemic hormonal counterregulation in individuals with a history of type 1 diabetes and impaired counterregulatory responses. In particular, these results showed that glucagon and epinephrine responses and hypoglycemic symptom recognition were not improved by islet transplantation.

Currently, one of the primary indications for islet trans-

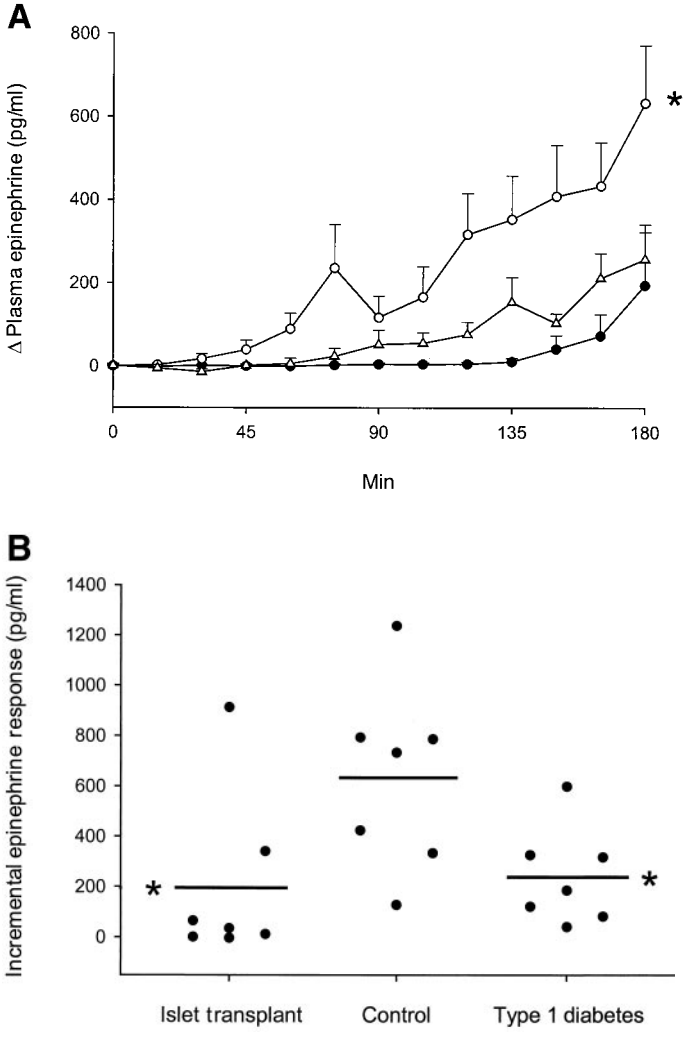


FIG. 4. *A*: Plasma epinephrine response during hypoglycemic clamp of islet transplant recipients (●), type 1 diabetic subjects (△), and nondiabetic control subjects (○). A significant rise in plasma epinephrine was observed in the control group. Two islet transplant recipients had elevations of plasma epinephrine, but overall there was no significant rise in mean plasma epinephrine in the islet transplant group or the type 1 diabetic group. *$P < 0.001$ vs. basal, ANOVA. *B*: Incremental epinephrine responses to hypoglycemia from baseline to 180 min of the islet transplant recipients (●), type 1 diabetic subjects (△), and nondiabetic control subjects (○). The mean incremental responses of the islet transplant recipients and the type 1 diabetic subjects did not differ significantly, and both were significantly less than that of the nondiabetic control subjects. *$P < 0.05$ vs. control, ANOVA.

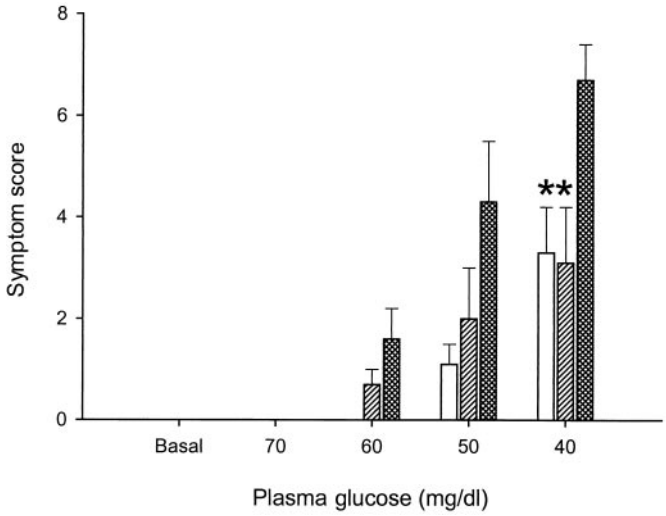


FIG. 5. Symptom scores of islet transplant recipients (□), type 1 diabetic subjects (▨), and nondiabetic control subjects (▩) at basal and 45-min plasma glucose levels during the hypoglycemic clamp. The scores of the islet transplant recipients did not differ significantly from those of the type 1 diabetic subjects at all glycemic levels. At 40 mg/dl (2.2 mmol/l), symptom recognition in both groups was significantly less than that observed in the nondiabetic control subjects. *$P < 0.05$ vs. control, ANOVA.

plantation is severe hypoglycemia unawareness, which often occurs in patients with long-standing type 1 diabetes. This unawareness puts them at risk for frequent, severe hypoglycemic events, such as seizure and coma (1,3,4). Patients using intensive insulin regimens are at particularly high risk (15–17). Strict avoidance of low blood glucose levels has been shown to restore symptom recognition and epinephrine responses in most diabetic patients with hypoglycemia unawareness (18–20). However, individuals with long-standing diabetes (>15 years) appear to be less likely to show improvement in epinephrine response and symptom recognition after a period of hypoglycemia avoidance than individuals with diabetes of shorter duration (21).

Studies of counterregulation in whole pancreas transplant recipients have shown both early (1 month post-transplant) and sustained (up to 19 years post-transplant) improvements in glucagon and epinephrine responses to hypoglycemia, as well as improvements in hypoglycemic symptom recognition (6–10). However, because pancreas transplantation carries the risks and morbidities of major surgery as well as long-term immunosuppression, its application outside of kidney transplantation remains limited (22). For type 1 diabetic patients who are at high risk for severe hypoglycemic events, but are not candidates for whole pancreas transplantation, intrahepatic islet transplantation has been proposed as a means of not only stabilizing blood glucose, but also allowing patients to potentially avoid severe episodes of hypoglycemia by improving hormonal counterregulation and symptom recognition. However, the current study suggests that this is not the case.

This is the first study to systematically examine counterregulatory responses at severe hypoglycemic levels (40 mg/dl) in a group of stable islet allotransplant recipients with prolonged insulin independence (mean post-transplantation duration >12 months). One previous study examined hormonal counterregulation in three islet allotransplant recipients before and 1 month after transplantation (23). Glucagon responses were not improved in any of the subjects, although epinephrine, norepinephrine, and cortisol responses were somewhat improved. However, two of the three subjects were not insulin independent at the time of the study, and the third became insulin independent over a period of 2 weeks, suggesting tenuous glycemic control. Also, the glycated hemoglobin values of all three subjects after transplantation were >8.0%, suggesting that post-transplant glycemic control in these individuals was suboptimal, thereby limiting the conclusions that could be drawn from the study.

Another study of two islet allotransplant and four islet autotransplant recipients with prolonged insulin indepen-

dence (23–107 months) found that glucagon responses of both auto- and allotransplant recipients did not improve after transplantation (24). However, the number of study subjects was small and the glucose nadir reached was only 55 mg/dl. Therefore, as with the previously discussed study, it is difficult to draw firm conclusions regarding the absence of α-cell responsiveness to hypoglycemia based on these results. In addition, the study did not examine epinephrine responses or hypoglycemic symptom recognition, both of which are important factors in protecting individuals with labile diabetes from severe hypoglycemia. An accompanying study suggested that impaired glucagon responsiveness might be related to the site of transplantation (25). This study found that the glucagon response was absent in pancreatectomized dogs with intrahepatic autotransplanted islets, but was intact in dogs with intraperitoneally transplanted islets. Thus it appears in this study that the failure of glucagon responsiveness was specific to the intrahepatic site.

The physiological explanation for such a defect remains unclear. Because the liver is the site of endogenous glucose production, higher intrahepatic glucose levels could potentially prevent transplanted islets from sensing peripheral hypoglycemia, thus causing an impairment of glucagon secretion. However, this explanation is not entirely satisfactory, as transplanted islets are presumably located on the portal venous side of the hepatic circulation, which tends to have a slightly lower ambient glucose level (25) and where it appears that an important glucosensor for the detection of hypoglycemia is located (26,27). More study is needed, therefore, if this apparent contradiction is to be resolved.

In the current study, the gradual reduction of the C-peptide levels observed in the islet transplant group during the clamp indicated an appropriate reduction of insulin secretion and verified that intrahepatic β-cells were able to respond to progressive hypoglycemia. Although there were no statistical differences in the C-peptide levels between the islet transplant and control groups, the levels appeared to be more reduced in the control group at lower plasma glucose values (i.e., <60 mg/dl). This finding raises the possibility that glucagon secretion in the islet transplant group may have been relatively more suppressed by insulin at glucose levels <60 mg/dl. However, as mentioned previously, a similar defect in glucagon secretion was found to exist in intrahepatic autoislet recipients who had C-peptide disappearance curves identical to those of the control group (24).

The robust glucagon response to arginine stimulation (Fig. 3*A*, inset) indicated the presence of functioning α-cells in the transplant recipients; however, this result does not differentiate between glucagon secreted by transplanted α-cells versus that secreted by the native pancreas. Clearly, glucagon secretion from native islets remained absent despite restoration of euglycemia.

Another potential cause of impaired α-cell function is toxicity of immunosuppressive drugs on transplanted islets. Tacrolimus has been shown to impair β-cell function and reduce insulin secretion in vitro and in vivo, but there is no clear evidence to show it impairs α-cell function in a similar fashion (28–31). Few studies have specifically examined the effect of sirolimus on α-cell function. However, there appears to be little effect of sirolimus on β-cell function in the setting of islet transplantation (32,33). More studies are needed before a firm conclusion can be reached regarding whether immunosuppressive toxicity plays a significant role in impaired α-cell function after islet transplantation.

In the current study, the epinephrine response of the islet transplant group also remained impaired after transplantation. This result was surprising, as a partial restoration of epinephrine response to hypoglycemia has been demonstrated in whole pancreas transplant recipients (9,10). In a study of pancreas transplant recipients who had autonomic insufficiency and poor symptom recognition, Kendall et al. (9) observed restoration of hormonal counterregulation and symptom recognition after transplantation.

As mentioned previously, strict avoidance of hypoglycemia in diabetic subjects has been shown to restore epinephrine responsiveness; however, individuals with long-standing diabetes appear to have a more modest recovery than individuals with diabetes of shorter duration (21). This may be the result of progressive diabetic autonomic neuropathy, which tends to be more significant in patients with long-standing diabetes (34,35). Because the mean duration of diabetes of the islet transplant recipients in the current study was 27 years, it is possible that underlying autonomic neuropathy was at least partially responsible for the lack of epinephrine response observed. However, because the current study was cross-sectional, comparisons of pre- and post-transplant autonomic function were not possible, so no firm conclusions regarding autonomic neuropathy can be drawn from these data.

Notably, two of the islet transplant recipients had significant rises in epinephrine during the study. One subject demonstrated a robust rise, whereas another had a more modest response. Because this was not a prospective study, it is impossible to know whether the epinephrine responses in these two subjects improved after transplantation. These two subjects also had the highest hypoglycemic symptom scores of the transplant group, including significant "adrenergic" symptoms such as tachycardia and perspiration, corresponding to an increase in plasma epinephrine. This correlation between symptoms and plasma hormone levels supports the proposition that the absence of epinephrine responses in the majority of islet transplant recipients is an accurate reflection of the counterregulatory state in these patients rather than an error in sample handling or assay technique.

Finally, symptom recognition did not improve after islet transplantation, even at very low plasma glucose levels (40 mg/dl). This result was also surprising, as pancreas transplantation and avoidance of hypoglycemia have both been shown to restore symptom recognition in diabetic subjects (9,10). As with epinephrine, the long duration of diabetes in this patient group may have made it less likely that they would recover symptom recognition after transplantation (21). Another explanation that may be considered is that despite apparent euglycemia, islet transplant patients could be subject to episodes of asymptomatic hypoglycemia after transplantation. As noted previously, the apparent lack of full suppression of C-peptide secretion at plasma glucose levels <60 mg/dl in the islet transplant group

suggests that insulin secretion may persist at these levels, which could put these patients at risk for unrecognized hypoglycemia, especially after meals. If insulin secretion persisted inappropriately in the islet transplant patients, then a significant recovery of epinephrine or symptom response would not necessarily be expected.

In summary, the current study found that despite prolonged insulin independence and near-normal glycemic control in islet transplant subjects, intrahepatic islet transplantation does not appear to restore hypoglycemic counterregulation or symptom recognition in subjects with long-standing diabetes and loss of symptom awareness. The absence of glucagon responsiveness suggests either an intrahepatic factor or an acquired defect in the ability of transplanted α-cells to sense or appropriately respond to hypoglycemia. Alternatively, an inadequate reduction of insulin secretion from transplanted islets could result in the suppression of glucagon responsiveness, possibly related to the site of implantation. If this is the case, alternative sites might conceivably improve physiological counterregulation. Also, the absence of the recovery of epinephrine and symptom recognition may reflect an underlying autonomic neuropathy of long-standing diabetes or repeated subclinical hypoglycemic episodes after transplantation. Finally, even though we have not observed clinically significant hypoglycemic episodes in insulin-independent islet recipients, the failure to restore counterregulatory responsiveness after islet transplantation may put transplant recipients who resume using exogenous insulin at risk for recurrent episodes of hypoglycemia.

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