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EARLY METABOLIC MARKERS OF ISLET ALLOGRAFT DYSFUNCTION

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Abstract

Background—Islet transplantation (IT) can restore normoglycemia to patients with unstable type 1 diabetes (T1DM) but long term insulin independence is usually not sustained. Identification of predictor(s) of islet allograft dysfunction (IGD) might allow for early intervention(s) to preserve functional islet mass.

Methods—Fourteen IT recipients with long-term history of T1DM underwent metabolic testing by mixed meal tolerance test (MMTT), intravenous glucose tolerance test (IVGTT), and arginine stimulation test (AST) every 3 months post islet transplant completion. Metabolic responses were compared between subjects who maintained insulin independence at 18 months (Group-1; n=5) and those who restarted insulin within 18 months (Group-2; n=9). Data was analyzed before development of islet graft dysfunction and while insulin independent.

Results—The 90min glucose, time-to-peak C-peptide, and AUC_{glucose} were consistently higher in Group-2 and increased as a function of time. At 12 months, acute-insulin-release to glucose (AIRg) in Group-2 was markedly reduced as compared to baseline ($5.62 \pm 1.21 \mu\text{IU/mL}$, n=4 vs. $16.14 \pm 3.69 \mu\text{IU/mL}$, n=8) whereas it remained stable in Group-1 ($22.36 \pm 4.98 \mu\text{IU/mL}$, n=5 vs. $27.70 \pm 2.83 \mu\text{IU/mL}$, n=5). AIRg, acute C-peptide release to glucose (ACpRg), and mixed meal stimulation index (MMSI) were significantly decreased and time-to-peak C-peptide, 90min glucose and AUC_{glucose} were significantly increased when measured at time-points preceding intervals where IGD occurred compared to intervals where there was no IGD.

Conclusions—The IVGTT and MMTT may be useful in the prediction of IGD and should be essential components of the metabolic testing of islet transplant recipients.

Keywords

Islet transplantation; type 1 diabetes; metabolic function; graft dysfunction

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INTRODUCTION

Islet transplantation (IT) can restore normoglycemia and improve the quality of life of patients with unstable type 1 diabetes mellitus (T1DM) (1). We have previously reported an insulin independence rate of 79% at 1 year (2), similar to the experience by the Edmonton group (3). However, despite sustained C-peptide levels, only a small percentage of patients (<10%) maintain insulin independence at 5 years (4). Several studies in islet allograft recipients have shown greatly reduced responses as compared to controls (2,3,5–8) suggesting a markedly reduced and/or dysfunctional islet mass. Many factors may play a role in leading to islet allograft dysfunction (IGD) including impaired engraftment (9), toxicity from the immunosuppressant agents (10,11), allo-rejection (12,13), recurrence of auto-immunity (14, 15) and β -cell metabolic exhaustion. The appearance of persistent hyperglycemia in a previously insulin independent normoglycemic islet allograft recipient is usually the outcome of an event(s) that led to dysfunction or loss of the islet mass rather than an indicator of an acute event. Any type of intervention at this point may prevent further dysfunction although it may be too late to recover whatever function was lost.

The detection of marker(s) that may predict IGD becomes crucial and could allow for early intervention(s) to preserve functional islet mass and maintain long-term insulin independence.

The aim of our study was to compare the metabolic responses between subjects who maintain insulin independence and those who eventually require reintroduction of insulin and to identify metabolic markers that may predict IGD.

RESEARCH DESIGN AND METHODS

Subjects

Sixteen subjects with long standing history of T1DM and hypoglycemia unawareness who underwent IT were recruited. The islet isolation, transplantation procedure and immunosuppressive regimen have been previously described (2). Briefly, immunosuppression consisted of maintenance starting at post operative day (POD)-1 with tacrolimus (Prograf®, Fujisawa, Japan) target trough level 4–6ng/ml and sirolimus (Rapamune®, Wyeth Pharmaceuticals Inc., Madison, NJ, USA), target trough level 12–15ng/mL for 3 months and 10–12ng/mL thereafter, and a 5 dose induction course of daclizumab (Zenapax®, Roche, Nutley, NJ, USA) 1mg/Kg IV biweekly beginning the day of transplant. Two patients who received a single infusion and required cessation of immunosuppression due to immunosuppression related adverse events are not included in the analysis. The research protocol was approved by the University of Miami health research ethics board (IRB) and each subject gave written informed consent.

Definitions

Insulin independence—C-peptide positive islet transplant recipients with capillary fasting and 2-h post-prandial glucose levels ≤ 140 mg/dL and ≤ 180 mg/dL, respectively and an HbA1c $\leq 6.5\%$ (16).

Graft dysfunction—C-peptide positive islet transplant recipients displaying fasting capillary glucose levels > 140 mg/dL and/or 2-h post-prandial capillary glucose levels > 180 mg/dL in three or more occasions in 1 week and/or two consecutive HbA1c values $> 6.5\%$ leading to reintroduction of insulin treatment.

Islet transplant completion—Insulin independence after either one or two islet infusions.

Metabolic testing

Subjects underwent metabolic testing after an overnight fast (8–12hrs) every 3 months post-islet transplant completion up to 18 months and every 6 months thereafter as long as graft function was maintained (detectable fasting C-peptide and/or stimulated C-peptide >0.3ng/ml). Tests were only conducted if capillary glucose was ≤ 140 mg/dl before the test. Plasma glucose concentrations were determined by the hexokinase method. Plasma C-peptide was measured by double antibody radioimmunoassay (detection limits: 0.3–5.0 ng/mL, inter- and intra-assay variation coefficient <10%, cross-reactivity with insulin and pro-insulin: 20%). Serum insulin was measured by a solid phase ^{125}I radioimmunoassay (DPC®, Los Angeles, CA) in a gamma counter (detection limits: 3.0–400.0 $\mu\text{IU/mL}$; inter-assay variation coefficient 4.5%; intra-assay variation coefficient 7.6%; cross-reactivity with pro-insulin: 20%).

a. Mixed meal tolerance test—MMTT was conducted on the first day. Subjects ingested 12 oz. of BOOST® High Protein (calories 360, total fat 9g, total carbohydrate 49.5g, total protein 22.5g) over a minute. Samples for glucose and C-peptide were obtained at 0, 15, 30, 60, 90, 120, 150, 180, 210, 240, 270 and 300 minutes following ingestion. We assessed the area under the curve for glucose ($\text{AUC}_{\text{glucose}}$) and C-peptide ($\text{AUC}_{\text{C-peptide}}$), mixed meal stimulation index (MMSI), 90-min glucose and C-peptide, peak glucose and C-peptide (defined as the highest glucose and C-peptide achieved during MMTT), and time-to-peak glucose and C-peptide. $\text{AUC}_{\text{glucose}}$ and $\text{AUC}_{\text{C-peptide}}$ were calculated using the trapezoidal rule and represent the area above baseline. The MMSI was calculated as follows: $\text{MMSI} = \frac{\text{AUC}_{\text{C-peptide}} (\text{pmol/mL})}{[\text{AUC}_{\text{glucose}} (\text{mg/dL})/100]}$ (17).

b. Intravenous glucose tolerance test—IVGTT was performed the day after the MMTT. A solution of 50% Dextrose (300mg/Kg) was infused over a minute and samples for glucose, C-peptide and insulin were obtained at –10, 0, 3, 4, 5, 7, 10, 15, 20, 25, 30, and 60 minutes. We measured the acute C-peptide release to glucose (ACpRg) and acute insulin release to glucose (AIRg). ACpRg and AIRg were defined as the average of c-peptide or glucose values at 3, 4 and 5 min minus the average of baseline values (3,18,19).

c. Arginine stimulation test—AST was performed one hour after the IVGTT. A dose of 5g of arginine hydrochloride (R-gene® 10, Pharmacia & Upjohn Company, NY, NY 10017) was infused over 30 seconds and samples were collected at –10, 0 (halfway through the arginine infusion), 2, 3, 4, 5, 7, and 10 min. We measured the acute C-peptide release to arginine (ACpRarg), and acute insulin release to arginine (AIRarg). ACpRarg and AIRarg were defined as the average of the 3 highest c-peptide or glucose values at 2, 3, 4 and 5 min minus the -10 min value (19,20).

C-PEPTIDE/GLUCOSE RATIO (CPGR)

The CPGR was obtained from fasting c-peptide and glucose values at each of the testing time-points and calculated as previously described (16).

HOMEOSTATIC MODEL ASSESSMENT (HOMA)

Insulin resistance was measured with the HOMA-IR and calculated as follows: $[\text{fasting insulin } (\mu\text{IU/mL}) \times \text{fasting glucose (mg/dL)}]/405$ (21).

Statistical analysis

Preliminary analysis: Assessing insulin independent subjects vs. subjects who required reintroduction of insulin—As a preliminary analysis of the data, we first assessed differences in the metabolic responses between subjects who remained insulin

independent at 18 months post-completion (Group-1) and those who required reintroduction of insulin within 18 months post-completion (Group-2). Five subjects (IA 4, 5, 9, 12 and 16) comprise Group-1 and nine subjects (IA 1, 3, 6, 7, 8, 10, 13, 14 and 15) comprise Group-2. Comparisons were made between groups at 3, 6, 9, 12 and 15 months to assess differences in each of the metabolic measures. At each time-point, comparisons were made between subjects in Group-2 who were still off insulin at that time-point vs. subjects in Group-1. Subjects in Group-2 were not considered in comparisons at time-points after they restarted insulin so that the data analyzed would be an appropriate reflection of islet graft function without the effect of exogenous insulin. Comparisons between both groups were only possible up to 15 months post-completion after which time all subjects in Group-2 had restarted insulin therapy. At each time-point under consideration, preliminary comparisons were made with two sample t-tests to assess differences in metabolic variables between groups. Values are expressed as means $\pm$ SE unless otherwise noted. Differences were considered statistically significant at a p value <0.05 .

Linear Mixed Model Regression Analysis: comparison of measures observed at time-points preceding intervals with dysfunction with measures observed at time-points preceding intervals without dysfunction—

Of primary interest in this investigation is to determine factors that may predict impending IGD. Thus, it is of interest to assess how metabolic measures may be different when observed at time-points preceding intervals where dysfunction occurs in comparison to measures observed at time-points preceding intervals without dysfunction. For each of the metabolic measures under consideration, we performed a repeated measures analysis of the data using linear mixed model regression. This method generalizes linear regression techniques to allow for repeated observations by taking into account the correlation that exists within observations on the same subject to more appropriately estimate variances used for the various tests of significance. In the regression model, a covariate was included for each observation which indexed if the outcome was measured at a time-point preceding the interval where the patient experienced IGD or not. Using this approach, we were able to simultaneously estimate and test differences in each of the metabolic measures under consideration between measures taken at time-points preceding intervals where dysfunction occurred and time-points preceding intervals where there was no dysfunction. This analysis considers data from every patient at every time-point where the patient has not developed IGD. Analysis was performed using SAS 9.1 software (SAS Institute Inc., Cary, NC, USA).

RESULTS

Subject Characteristics

Fourteen subjects (7 males and 7 females) with a mean age of 43 ± 2 years and duration of diabetes of 29 ± 3 years who underwent IT at our Institution between February 2001 and July 2003 were evaluated (Table-1). Subjects received an average of $13,633 \pm 804$ IEQ/Kg and all achieved insulin independence, 13 after 2 islet infusions and 1 (IA 9) after a single infusion. Five subjects who remained insulin free at 18 months (IA 4, 5, 9, 12 and 16) were assigned to Group-1 and the remaining 9 subjects (IA 1, 3, 6, 7, 8, 10, 13, 14 and 15) were assigned to Group-2. Subjects in Group-2 were progressively removed from analysis as they restarted insulin. Diabetes duration was longer in Group-2, 33 ± 3 years vs. 20 ± 4 years, $p=0.023$ (Table-1). Seven out of 9 subjects (78%) in Group-2 had a history of microvascular complications as compared to 2 out of 5 subjects (40%) in Group-1. Group-2 received more IEQ/Kg, $15,035 \pm 649$ IEQ/Kg vs. $11,108 \pm 1,376$ IEQ/kg, $p=0.012$. Group-1 developed IGD at a median of 1,194 days post-completion (range 679–1,397) and restarted insulin at a median of 1,197 days (range 828–1454), whereas Group-2 developed IGD at a median of 263 days post-completion (range 42–451) and restarted insulin at a median of 380 days (range 167–471).

Mixed Meal Tolerance Test

Group-1, insulin independent subjects at 18 months post-completion—At 3 months (baseline), basal glucose was $98 \pm 3 \text{ mg/dL}$ with a C-peptide of $1.19 \pm 0.09 \text{ ng/mL}$ ($n=5$). The 90-min glucose was $103 \pm 1 \text{ mg/dL}$ with a C-peptide of $2.42 \pm 0.28 \text{ ng/mL}$ (Table-2). Peak glucose was $139 \pm 6 \text{ mg/dL}$ and peak C-peptide $2.98 \pm 0.18 \text{ ng/mL}$ occurring at 42 ± 7 and $66 \pm 15 \text{ min}$, respectively. MMSI was $2.82 \pm 0.41 \text{ pmol/mg}$. Subjects achieved a $2.53 \pm 0.19 \text{ ng/mL}$ C-peptide increase from basal ($n=5$) at 3 months which persisted by 18 months ($2.57 \pm 0.28 \text{ ng/mL}$, $n=4$).

At 18 months, 90-min glucose was significantly higher as compared to baseline, $148 \pm 8 \text{ mg/dL}$ ($n=5$) vs. $103 \pm 1 \text{ mg/dL}$ ($n=4$), $p=0.016$ (Table-2), as well as peak glucose, 162 ± 7 ($n=4$) vs. $139 \pm 6 \text{ mg/dL}$ ($n=5$), $p=0.04$ (Fig. 1). By contrast, fasting glucose was not significantly higher, 103 ± 6 ($n=4$) vs. $98 \pm 3 \text{ mg/dL}$ ($n=5$), $p=0.41$. Time-to-peak glucose and C-peptide were both delayed to $75 \pm 9 \text{ min}$ ($n=4$; $p<0.05$) and $98 \pm 14 \text{ min}$ ($n=4$; $p=NS$), respectively. The MMSI, which had consistently remained above 2.0, declined to 1.83 ± 0.66 (Table-2).

Group-2, subjects restarting insulin treatment within 18 months post-completion—Fasting glucose levels were consistently in the impaired fasting glucose range and significantly higher than Group-1 at baseline, 3 and 6 months ($p<0.01$) (Table 2). Basal C-peptide levels were also higher at 3 months ($1.85 \pm 0.24 \text{ ng/mL}$, $n=9$) although not significantly higher than Group-1 ($1.19 \pm 0.09 \text{ ng/mL}$, $n=5$; $p=0.08$) and remained comparable between groups thereafter (Fig. 1). Time-to-peak C-peptide was $110 \pm 14 \text{ min}$ at baseline ($n=9$), remaining higher than Group-1 and practically unchanged over time (Fig. 1). From 6–15 months, Group-2 had significantly higher 90-min glucose, peak glucose and $\text{AUC}_{\text{glucose}}$ than group-1 (Table 2). Similarly, 90-min C-peptide and peak C-peptide were significantly higher at baseline and remained higher than Group-1 (Fig. 1). MMSI was higher than Group-1 at baseline (6.33 ± 2.46 , $n=9$, vs. 2.82 ± 0.41 , $n=5$; $p=NS$) but decreased to 1.68 ± 0.21 by 6 months and to <1.0 by 15 months (0.67 ± 0.2 , $n=2$). Peak C-peptide increase from basal was $2.19 \pm 0.18 \text{ ng/mL}$ ($n=9$) at 3 months and it was maintained throughout follow up with a $2.76 \pm 0.28 \text{ ng/mL}$ ($n=3$) and $2.55 \pm 0.18 \text{ ng/mL}$ ($n=2$) increase at 12 and 15 months respectively.

Intravenous glucose tolerance test

Fasting glucose at baseline was similar in both groups, $103 \pm 5 \text{ mg/dL}$ in Group-1 ($n=5$) and $104 \pm 4 \text{ mg/dL}$ in Group-2 ($n=9$). Group-1 showed an initial AIRg of $27.70 \pm 2.83 \mu\text{IU/mL}$ and maintained stable responses for the first 15 months demonstrated by a fasting glucose of $92 \pm 5 \text{ mg/dL}$ and AIRg of $22.62 \pm 4.52 \mu\text{IU/mL}$ ($n=5$). A significant reduction in AIRg as compared to baseline was seen at 18 months, $15.29 \pm 4.46 \mu\text{IU/mL}$ ($n=5$) vs. 27.70 ± 2.83 ($n=5$) ($p=0.047$) whereas mean fasting glucose was almost unchanged at $104 \pm 6 \text{ mg/dL}$ ($n=5$). By contrast, Group-2 started with a markedly lower AIRg compared to Group-1 (16.14 ± 3.69 , $n=8$, vs. $27.70 \pm 2.83 \mu\text{IU/mL}$, $n=5$; $p=0.048$) (Figure 2), despite having similar mean fasting glucose values, and the AIRg continuously declined with time. By 12 months, fasting glucose was $110 \pm 10 \text{ mg/dL}$ and the AIRg had decreased to $5.62 \pm 1.21 \mu\text{IU/mL}$ ($n=4$), a 65% reduction as compared to baseline (Fig. 2). Data for a single patient in Group-2 was available for analysis at 15 months and showed a fasting glucose of 131 mg/dL with a suppressed AIRg of $1.31 \mu\text{IU/mL}$ (Fig. 2).

Initial ACpRg was $0.99 \pm 0.14 \text{ ng/mL}$ in Group-1 ($n=5$), remaining unchanged until 15 months ($0.84 \pm 0.20 \text{ ng/mL}$, $n=4$) with a slight reduction to $0.70 \pm 0.19 \text{ ng/mL}$ ($n=5$) by 18 months. Group-2 had an ACpRg at baseline of $0.70 \pm 0.11 \text{ ng/mL}$ ($n=9$), almost identical to the response in Group-1 at 18 months. ACpRg in Group-2 declined as a function of time reaching a value of $0.37 \pm 0.09 \text{ ng/mL}$ ($n=4$) by 12 months.

Arginine Stimulation Test

Both groups showed similar responses at baseline, with an AIRg of $24.55 \pm 6.73 \mu\text{IU/mL}$ and ACpRg of $0.89 \pm 0.09 \text{ ng/mL}$ ($n=5$) in Group-1 and an AIRg of $20.66 \pm 2.30 \mu\text{IU/mL}$ and ACpRg of $0.82 \pm 0.08 \text{ ng/mL}$ ($n=9$) in Group-2 (Fig. 2). Basal glucose was higher in Group-2 at all time points and significantly so at 6 ($p=0.03$) and at 9 months ($p=0.01$). However, AIRg responses remained stable and were not significantly different between groups at any time point (Table 3).

CPGR

CPGR in Group-1 was 1.21 ± 0.06 at 3 months ($n=5$). Responses remained consistently above 1.00 and practically unchanged at 18 months (1.14 ± 0.08 ; $n=5$). In Group-2, CPGR at baseline was 1.63 ± 0.21 ($n=9$), slightly higher than in Group-1 ($p=NS$). It declined by 12 months to 0.99 ± 0.11 ($n=5$) and remained unchanged by 15 months (1.00 ± 0.16 ; $n=2$).

HOMA IR

HOMA-IR was comparable between both groups at 3 months, 1.88 ± 0.12 ($n=5$) in Group-1 vs. 1.59 ± 0.16 ($n=9$) in Group-2. Group-1 showed a significant reduction by 6 months to 1.0 ± 0.15 ($n=5$; $p=0.002$) and this reduction was maintained by 15 months. By contrast, HOMA-IR in Group-2 remained stable for the first 9 months but increased by 12 months and was significantly higher than in Group-1, 1.99 ± 0.47 ($n=5$) vs. 0.79 ± 0.05 ($n=5$), $p=0.03$.

Correlation between IEQ/kg and metabolic markers

All measured stimulated MMTT C-peptide responses at baseline correlated with total IEQ/Kg with the highest correlation coefficient given by the 90 minute C-peptide ($r=0.643$, $p<0.001$; $n=14$). There was a positive correlation with the basal glucose from the MMTT at baseline and the total IEQ/Kg transplanted ($r=0.627$, $p<0.01$; $n=14$). Neither the AIRg nor the AIRarg correlated with the transplanted IEQ/Kg when measured at 3 months.

Linear Mixed Model Regression Analysis

The AIRg, ACpRg, and MMSI are significantly decreased when measured at post-transplant time-points preceding intervals where dysfunction occurred compared to intervals where there was no dysfunction. Similarly, 90 min glucose, time-to-peak C-peptide, and $\text{AUC}_{\text{glucose}}$ from the MMTT are significantly increased when measured at post-transplant time-points preceding intervals where dysfunction occurred compared to post transplant time-points preceding intervals where there was no dysfunction (Table-3). Additionally, analysis reveals a significant association between basal glucose and AIRg. A 10mg/dL increase in basal glucose is significantly associated with a 3.38 unit decrease in AIRg ($p<0.0001$).

DISCUSSION

Despite normalization of glycemic control and achievement of insulin independence, β -cell secretory responses to glucose and non-glucose secretagogues are significantly impaired in islet transplant recipients (2,3,5,7,8). Recipients of intrahepatic islet auto-transplants also display markedly reduced metabolic responses with the difference that in this setting insulin independence with near-normoglycemia can be maintained in many cases for over 10 years (22). However, as differs to islet auto-transplantation, islet allografts are exposed to several factors that can lead to dysfunction and/or loss of the β -cell mass including allojection, recurrence of autoimmunity, and toxicity from the immunosuppressive drugs which likely account for the reduced long term insulin independence rates (4,23).

The subjects reported herein demonstrated markedly reduced responses when compared to control data available in the literature (3,5,7,19). Clear differences were detected at baseline between the metabolic responses of both groups. Group-2 showed higher basal glucose, basal C-peptide and peak C-peptide during the MMTT. These findings may suggest an inadequate islet mass in Group-2 leading to a compensatory increase in β -cell function to maintain euglycemia. Insulin resistance may have also explained these results but we did not find significant differences in HOMA-IR values between groups at baseline. In addition, Rickels et al demonstrated that islet transplantation on sirolimus-tacrolimus maintenance immunosuppression led to an improvement on insulin sensitivity as compared to subjects with T1DM (24).

Group-2 also demonstrated a progressive increase in the 90min glucose with a concomitant increase in AUC_{glucose} and MMSI during a MMTT. All these variables proved to be significantly associated with development of IGD. The AUC_{glucose} during oral glucose tolerance testing (OGTT) has been described as significantly more predictive of type 1 diabetes than the 2-hr glucose in ICA-positive relatives of type 1 diabetic patients (25).

Despite a progressive deterioration in glucose levels during the MMTT as well as a slight decline with time in basal C-peptide in Group-2, both groups were able to maintain an almost identical increase, consistently >2 fold, in stimulated C-peptide during the MMTT suggesting that despite alterations in C-peptide production in the basal state, transplanted β -cells retain the ability to double their secretory capacity in response to ambient glucose. Interestingly, Group-2 developed IGD earlier than Group-1 despite receiving more IEQ/Kg. One possible explanation for this observation could be errors in the islet count since islet enumeration currently remains highly subjective. Another possibility is changes in the hepatic microvasculature. Group-2 had a longer duration of diabetes and a higher incidence of microvascular complications as compared to Group-1. The role of chronic diabetes in liver disease has been described and diabetic microangiopathy has been associated with liver sinusoidal abnormalities (26,27). The longer duration of diabetes in Group-2 may have contributed to alterations in the hepatic vascular bed leading to problems with islet engraftment.

Abnormalities in the first phase of insulin secretion have been well documented in the pre-diabetic stage of T1DM (28,29) and a loss of first phase is the classical finding at diagnosis of type 2 diabetes mellitus (T2DM) (30,31). This leads to an abnormal regulation of post prandial glucoses resulting in post prandial hyperglycemia and it is usually the result of a qualitative defect of the β -cell, a markedly reduced islet mass, or a combination of both. We observed markedly reduced AIRg responses at baseline in both groups with significantly lower responses in Group-2 suggesting an already dysfunctional and likely lower engrafted mass in these subjects. Brunzel et al evaluated the insulin secretion and glucose disappearance rate in 66 subjects with a wide range of fasting plasma glucose and reported that the AIRg is consistently absent when fasting glucose levels exceed 115mg/dL (32). However, similar data are not available for islet transplant subjects who are insulin independent by islet transplant criteria (16). We observed that in the few subjects with fasting glucose levels between 116 and 140mg/dL, a reduced AIRg was still present. Monitoring of these AIRg responses proved to be a significant predictor of IGD.

In the setting of T2DM, despite a loss of the first phase of insulin release to glucose, β -cells are still able to respond to non-glucose secretagogues like arginine suggesting primarily a qualitative defect in the glucose sensing ability of the β -cell (31). Both groups maintained comparable AIRarg responses, demonstrating that transplanted islets also tend to first lose their glucose sensing properties but maintain responses to non-glucose secretagogues. It is possible that in the setting of a markedly reduced first phase of insulin release and a stable preserved AIRarg, a qualitative defect may be the primary problem rather than a reduced islet mass but

this remains to be demonstrated. All MMTT stimulated C-peptide responses at baseline correlated with total IEQ/Kg transplanted but we found no correlation with the AIRg or AIRarg. Paradoxically, there was a positive correlation with the basal glucose from the MMTT and the total IEQ/Kg transplanted ($r=0.627$, $p<0.01$; $n=14$) which further raises the possibility of errors in islet enumeration and stresses the need for objective islet counting techniques.

CONCLUSIONS

Marked metabolic abnormalities are evident early post IT in subjects who eventually will develop IGD. Monitoring of the 90min glucose, time-to-peak C-peptide, AUC_{glucose} , and MMSI from a MMTT and the AIRg and ACpRg may predict the onset of IGD.

Simple tests such as the IVGTT and MMTT provide adequate graft function information and may be useful in the prediction of IGD making them essential components of the metabolic testing of islet transplant recipients.

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ABBREVIATIONS

IT	Islet transplantation
T1DM	type 1 diabetes mellitus
IGD	islet allograft dysfunction
MMTT	mixed meal tolerance test
MMSI	mixed meal stimulation index
AUC_{gluc}	Area under the curve for glucose
$AUC_{\text{C-peptide}}$	Area under the curve for C-peptide
IVGTT	intravenous glucose tolerance test
AIRg	Acute insulin release to glucose
ACpRg	Acute C-peptide release to glucose
AST	Arginine stimulation test

AIRarg

Acute insulin release to arginine

ACpRarg

Acute C-peptide release to arginine

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Figure 1. Mixed Meal Tolerance Test glucose and C-peptide responses for Group-1 (A and B) and Group-2 (C and D).

Figure 2.

Metabolic variables from the IVGTT (AIRg), AST (AIRarg) and MMTT (90min glucose and AUCglucose) separated by groups. The dark gray bars represent Group-1 and the light gray bars represent Group-2. Numbers above bars represent the sample size. A drop out of patients occurs due to reintroduction of insulin, problems with peripheral IV access, venous sampling or conditions that precluded the patient from undergoing the test at that time. * $p < 0.05$ Group-1 vs. Group-2 (t-test).

Table 1

Baseline characteristics

Subject ID	Gender	Age (years)	Weight (Kg)	BMI (Kg/m ²)	T1DM duration (years)	Insulin use (U/Kg/day)	HbA1c (%)	Creatinine Clearance (ml/min/1.73m ²)	Islet infusions	Total IEQ/Kg	Insulin independence POD ^a	Graft dysfunction POD ^a	Insulin restarted POD ^a
GROUP-1 (n=5)													
IA4	F	32	68	25.7	19	0.47	9.4	128	2	13,324	1	1197	1,197
IA5	M	37	74	24.7	14	0.47	7.3	124	2	12,956	1	1397	1,454
IA9	M	35	97	27.4	17	0.33	8.3	103	1	6,251	45	679	1,013
IA12	M	51	88	25.7	39	0.48	7.0	173	2	9,823	0	1194	1,308
IA16	F	40	60	23.4	12	0.55	7.1	125	2	13,185	33	822	828
Mean±SE		39±3	77±7	25.5±0.8	20±4	0.46±0.04	7.8±0.5	131±11		1,1108±1,376	16±9	1,058±133	1,160±110
GROUP-2 (n=9)													
IA1	M	36	79	23.5	10	0.5	7.5	142	2	18,028	1	185	257
IA3	F	40	66	23.5	31	0.5	7.2	96	2	13,421	1	331	380
IA6	F	43	57	23.7	37	0.54	7.7	119	2	18,403	1	139	186
IA7	F	43	70	25.7	30	0.56	9.4	82	2	14,487	1	263	398
IA8	M	36	69	26.6	34	0.58	8.0	129	2	13,795	1	431	471
IA10	M	36	66	26.3	34	0.53	6.8	102	2	15,590	1	451	454
IA13	M	59	86	26.8	46	0.57	8.3	88	2	14,728	1	42	167
IA14	F	52	53	20.8	44	0.29	7.6	81	2	13,579	1	198	182 ^b
IA15	F	55	59	23.1	35	0.32	6.3	75	2	13,288	1	343	401
Mean±SE		44±3	67±3	24.4±0.7	33±3	0.49±0.04	7.6±0.3	102±8		15035±649	1	265±46	339±40
<i>p</i> ^c		0.302	0.158	0.344	0.023	0.636	0.72	0.053		0.012		<0.001	<0.001

^aPOD = Post operative day after completion.

^bPatient IA14 resumed insulin 16 days prior to development of ICGD without notifying the clinical program.

^cGroup-1 vs. Group-2. Unpaired 2 tailed t-tests.

Table 2
MMTT metabolic variables up to 18 months post completion.

Months post completion	3	6	9	12	15	18
Group-1						
(n)	(5)	(5)	(5)	(5)	(4)	(4)
Baseline glucose (mg/dL)	98±3	99±2	98±1	97±5	98±4	103±6
90min glucose (mg/dL)	103±11	79±7	113±4	96±6	121±10	148±8
AUC _{glucose} (mg/dL min)	3,002±1,078	1,266±377	2,199±370	2,486±190	3,103±681	4,714±999
AUC _{C-peptide} (ng/ml min)	215±52	218±45	222±39	219±30	232±43	225±56
MMSI (pmol/mg)	2.82±0.41	7.61±2.00	3.81±0.93	3.10±0.65	3.14±1.21	1.83±0.66
Group-2						
(n)	(9)	(7)	(6)	(3)	(2)	-
Baseline glucose (mg/dL)	113±2	114±3	111±2	110±3	114±19	-
90min glucose (mg/dL)	131±10	153±17	174±19	183±17	239±18	-
AUC _{glucose} (mg/dL min)	3,282±793	7,594±1,990	7,737±1,832	9,008±1,908	15,049±4,789	-
AUC _{C-peptide} (ng/ml min)	270±36	343±50	247±30	329±63	272±4	-
MMSI (pmol/mg)	6.33±2.46	1.68±0.21	1.69±0.41	1.30±0.34	0.67±0.20	-
p (Group-1 vs. Group-2)						
Baseline glucose (mg/dL)	0.001	0.004	<0.001	0.113	0.289	-
90min glucose (mg/dL)	0.102	0.008	0.019	0.001	0.003	-
AUC _{glucose} (mg/dL min)	0.837	0.025	0.024	0.004	<0.001	-
AUC _{C-peptide} (ng/ml min)	0.391	0.107	0.618	0.121	0.569	-
MMSI (pmol/mg)	0.319	0.005	0.053	0.059	0.246	-

Table 3
Basal glucose and acute insulin responses during Arginine Stimulation Test.

Months post completion	3	6	9	12	15	18
Group-1						
n	(5)	(5)	(3)	(3)	(5)	(4)
Baseline glucose (mg/dL)	101±7	92±6	94±5	104±4	92±9	110±2
AIIRarg (uIU/ml)	24±6.49	21.55±1.2	28.06±8.03	25.74±7.5	24.09±2.99	28.73±2.06
Group-2						
n	(9)	(5)	(5)	(2)	-	-
Baseline glucose (mg/dL)	117±5	119±8	121±5	119±1	-	-
AIIRarg (uIU/mL)	19.78±2.21	27.07±7.19	26.79±8.68	19.22±1.75	-	-
p (Group-1 vs. Group-2)						
Baseline glucose (mg/dL)	0.08	0.027	0.012	0.06	-	-
AIIRarg (uIU/mL)	0.43	0.47	0.93	0.55	-	-

Table 4

Linear Mixed Model Regression Analysis.

Metabolic variables	Estimated mean at timepoints preceding intervals with dysfunction	Estimated mean at timepoints preceding intervals with no dysfunction	Differences between time points preceding intervals with dysfunction and time points preceding intervals with no dysfunction	<i>p</i>
Fasting glucose	110.45±3.65	103.96±2.36	-6.49±3.68	0.077
Fasting C-peptide	1.37±0.11	1.46±0.12	0.09±0.10	0.336
Glucose 90min	153.07±6.54	123.91±6.62	-29.16±7.47	<0.0001
C-peptide 90min	3.00±0.14	3.12±0.19	0.12±0.16	0.427
Peak glucose	170.05±5.51	154.50±5.50	-15.55±5.89	0.008
Peak C-peptide	3.24±0.15	3.47±0.17	0.22±0.13	0.093
Time to peak glucose	57.56±3.96	54.93±4.10	-2.63±6.62	0.692
Time to peak C-peptide	119.1±9.6	91.5±8.1	-27.6±11.4	0.014
Glucose AUC	6,645.14±843.69	3,607.49±453.30	-3,037.65±628.53	<0.0001
C-peptide AUC	275.62±32.53	268.25±24.53	-7.37±27.29	0.787
MMSI	1.60±0.20	3.67±0.60	2.07±0.54	0.0001
AIRg	10.31±2.23	20.50±2.64	10.19±2.42	<0.0001
ACpRg	0.52±0.09	0.82±0.09	0.30±0.08	<0.001
AIRarg	21.58±3.15	24.42±2.47	2.84±2.19	0.196
ACpRarg	0.96±0.07	0.88±0.08	-0.07±0.05	0.16
CPGR	1.23±0.08	1.38±0.09	0.15±0.08	0.061
A1c	5.88±0.07	5.75±0.07	-0.14±0.11	0.229
HOMA IR	1.64±0.23	1.33±0.09	-0.30±0.22	0.175