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Early Metabolic Markers That Anticipate Loss of Insulin Independence in Type 1 Diabetic Islet Allograft Recipients

D. Hirsch[a], **J. Odorico**[a], **J. S. Danobeitia**[a], **R. Alejandro**[b], **M. R. Rickels**[c], **M. Hanson**[a], **N. Radke**[a], **D. Baidal**[b], **D. Hullett**[a], **A. Naji**[d], **C. Ricordi**[e], **D. Kaufman**[a], and **L. Fernandez**[a,*]

[a]Department of Surgery, Division of Transplantation, University of Wisconsin, Madison, WI

[b]Department of Medicine, Division of Endocrinology, University of Miami School of Medicine, Miami, FL

[c]Department of Medicine, Division of Endocrinology, Diabetes and Metabolism, University of Pennsylvania School of Medicine, Philadelphia, PA

[d]Department of Surgery, Division of Transplantation, University of Pennsylvania School of Medicine, Philadelphia, PA

[e]Department of Surgery, Microbiology and Immunology, University of Miami, Miami, FL

Abstract

The objective of this study was to identify predictors of insulin independence and to establish the best clinical tools to follow patients after pancreatic islet transplantation (PIT). Sequential metabolic responses to intravenous (I.V.) glucose (I.V. glucose tolerance test [IVGTT]), arginine and glucose-potentiated argi-nine (glucose-potentiated arginine-induced insulin secretion [GPAIS]) were obtained from 30 patients. We determined the correlation between transplanted islet mass and islet engraftment and tested the ability of each assay to predict return to exogenous insulin therapy. We found transplanted islet mass within an average of 16 709 islet equivalents per

*Corresponding author: Luis A. Fernandez, Luisf@surgery.wisc.edu.

Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Figure S1: Correlation analysis between SUITO index (fasting C-peptide [ng/mL]/[fasting blood glucose (mg/dL) – 63] × 1500) and (A) relative insulin requirement (ratio between pretransplant and posttransplant daily insulin requirements at 12 months), (B) relative HbA1c level (ratio between pretransplant and 12-month posttransplant HbA1c level), (C) blood glucose (AUC first phase) during IVGTT at 12 months posttransplant, (D) insulin release (AUC first phase) during IVGTT at 12 months posttransplant and (E) C-peptide release (AUC first phase) during IVGTT at 12 months posttransplant. Data shown represent slope and 95% confidence intervals for each data set. Statistical significance was considered at $p < 0.05$.

Figure S2: (A) SUITO index calculated at 3, 6 and 12 months posttransplant as a predictor of insulin independence at 12 months. Data are mean ± SEM. Nonparametric t-test used to establish differences between groups. A p-value < 0.05 was considered significant. (B) Receiver operator characteristic analysis of SUITO Index as a predictor of insulin independence at 12 months posttransplant. The ROC graph recorded a point for each data pair (clinical outcome) as if it was the critical value for a predictive assay and considering the data set at that point as true positives and false positives. Area under the ROC curve was then calculated. Tests that cannot discriminate between true and false positives show a sensitivity plot that is not significantly different from the line of identity and a p-value > 0.05 when the AUC is calculated. Cutoff values that generate maximized likelihood ratios for each assay are shown along with the sensitivity and specificity of the assay using that cutoff and the AUC for the plotted ROC graph for the assay.

Table S1: Descriptive statistics of pancreas donor demographics. Data are mean ± SEM and range where applicable

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kg body weight (IEQ/kg BW; range between 6602 and 29 614 IEQ/ kg BW) to be a poor predictor of insulin independence at 1 year, having a poor correlation between transplanted islet mass and islet engraftment. Acute insulin response to IVGTT (AIR_{GLU}) and GPAIS (AIR_{max}) were the most accurate methods to determine sub-optimal islet mass engraftment. AIR_{GLU} performed 3 months after transplant also proved to be a robust early metabolic marker to predict return to insulin therapy and its value was positively correlated with duration of insulin independence. In conclusion, AIR_{GLU} is an early metabolic assay capable of anticipating loss of insulin independence at 1 year in T1D patients undergoing PIT and constitutes a valuable, simple and reliable method to follow patients after transplant.

Keywords

Clinical islet transplantation; insulin independence

Introduction

Pancreatic islet transplantation (PIT), a minimally invasive β-cell replacement approach for patients with type 1 diabetes (T1D) complicated by hypoglycemic unawareness, can restore near-normal glycemic control and alleviate severe hypoglycemic episodes. Although the currently accepted therapeutic approach of transplanting islets isolated from more than one donor pancreas has resulted in insulin independence for many recipients, the majority return to some insulin requirement even when persistent islet graft function is evident by C-peptide levels.

Assessment of β-cell secretory capacity from glucose potentiation of insulin or C-peptide release in response to a nonglucose secretagogue such as arginine is the most accurate method to determine functional islet β-cell mass in humans (1). Testing of β-cell function by measuring insulin or C-peptide responses to intravenous (I.V.) glucose (AIR_{GLU} or ACR_{GLU}) or arginine (AIR_{ARG} or ACR_{ARG}) alone has been used as a surrogate measure for β-cell secretory capacity in islet recipients (2,3). A clearer understanding of initial and long-term islet β-cell engraftment is necessary to improve long-term insulin independence.

To date, metabolic studies in PIT have revealed a markedly impaired first-phase insulin response to AIR_{GLU} (4–6), a less impaired response to AIR_{ARG} (7) and a dramatically blunted β-cell response to glucose potentiation of arginine-induced secretion (GPAIS; AIR_{MAX}; Refs. 8,9). AIR_{GLU} is lost before AIR_{ARG} during β-cell mass reductions (10), likely because increases in fasting glucose desensitize β-cell response to glucose stimulation but potentiate β-cell response to arginine (11). As a surrogate measure for AIR_{MAX}, AIR_{GLU} has been considered a simple and sensitive indicator to assess early islet graft impairment, whereas AIR_{ARG} has been considered a more accurate test to predict surviving islet β-cell mass (3,7).

The purpose of this comparison was to establish predictors of insulin independence after PIT. To address this, sequential metabolic testing at 3, 6 and 12 months posttrans-plant was performed in 30 PIT recipients transplanted at three different institutions and the results

compared to 10 matched control subjects to evaluate β-cell responsiveness to glucose, arginine and GPAIS.

This study also addresses four clinically important questions in the field of islet transplantation:

1. What is the metabolic impairment (β-cell secretory capacity) of insulin-independent PIT recipients versus well-matched nondiabetic controls?
2. Does transplanted islet mass correlate with insulin independence at 1 year?
3. Can insulin and C-peptide secretion be used as a clinical tool to predict subsequent exogenous insulin requirement?
4. Do patients who remain insulin independent 1 year after PIT have a greater engrafted islet mass than patients returning to insulin within 1-year posttransplant?

Materials and Methods

Subjects

Potential islet recipients (18–65 years; T1D > 5 years) were recruited using standard inclusion/exclusion criteria: (http://www.fda.gov/biologicsbloodvaccines/guidancecomplianceregulatoryinformation/guidances/cellularandgenetherapy/ucm182440.htm). Inclusion criteria consisted of T1D with labile diabetes manifested by hypoglycemic unawareness complicated by frequent severe hypoglycemic episodes, recurrent ketoacidosis or already on immunosuppression for an existing kidney transplant. Thirty T1D subjects with longstanding C-peptide-negative disease were listed for PIT at the University of Miami, University of Pennsylvania and University of Wisconsin (UW; Table 1). Twenty-four patients underwent islet transplant alone (ITA) and six islet after kidney (IAK). All received immunosuppressive therapy based on modifications to the previously published Edmonton protocol (12). Briefly, IL-2 receptor blockade (1 mg/kg every 14 days for five consecutive doses) was given at transplant and steroid-free immunosuppression maintenance using tacrolimus and sirolimus (2–5 ng/mL and 10–14 ng/mL, respectively) for the first year. ITA and IAK recipients trough levels were managed similarly using sirolimus (8–12 ng/mL) and calcineurin inhibitors (tacrolimus 2–5 ng/mL). Four patients with well-functioning kidney allografts were corticosteroid-free for at least 9 months at enrollment. The additiona four patients received prednisone maintenance therapy of ≤5 mg/day. Patients receiving maintenance therapy with mycophenolate mofetil were maintained without dose modification. Eight subjects received a single dose of infliximab (Remicade®, Centocor, Malvera, PA, USA; 5 mg/kg), 2 h before first infusion.

Subjects were asked to record their daily insulin dose in self-monitoring diaries. Insulin dependence was defined as need for exogenous insulin to maintain HbA1c ≤ 6.5% and receiving exogenous insulin to maintain fasting capillary glucose level ≤140 mg/dL (7.8 mmol/L) at a minimum of 4 of 7 days per week, with 2-h postprandial capillary glucose levels not exceeding 180 mg/dL (10.0 mmol/L) more than three times per week. Subjects not receiving insulin who had HbA1c ≤ 6.5% with fasting and 2-h postprandial within target were deemed insulin independent. Ten controls matched for BMI, gender and age included

seven historical controls (13) and three contemporaneous controls. Metabolic testing was approved by UW, Miami and Penn Health Sciences Institutional Review Boards, with written informed consent obtained from all subjects.

Islet monitoring: tests of β -cell function and secretory capacity

Most patients were monitored after islet transplantation using a similar follow-up protocol by the three different institutions. Patients were followed at transplant clinic at 2, 4, 8 and 12 weeks after each infusion and followed every 3 months. The timing of follow-up assessments was "reset" with additional transplants. Stimulation tests using glucose and/or arginine as secretagogues measuring insulin and C-peptide responses and changes over time were compared between islet recipients and controls. Sequential metabolic testing was performed at 3, 6 and 12 months post last islet infusion. Briefly, subjects fasted overnight before testing. Insulin-dependent subjects withheld long-acting insulin for 24 h and short-acting insulin for 12 h before testing. If necessary, I.V. insulin was administered overnight to maintain blood glucose concentration <7 mM and discontinued ≥45 min before testing. On the morning of the test, one additional catheter was placed in the contralateral hand vein for blood sampling and the hand placed in a thermoregulated box (50°C) to promote optimal arterialization of venous blood. Thirty patients were available at 3 months, 19 at 6 months and 27 at 12 months posttransplant. At each time point, blood samples were collected for glucose, insulin and C-peptide analysis. Plasma glucose was measured immediately using a YSI 2300 Stat Glucose Analyzer (Yellow Springs Instruments, Yellow Springs, OH, USA). Additional serum or plasma was collected for determination of immunoreactive insulin and C-peptide concentrations by commercial assays (Millipore, Bellerica, MA, USA). The samples were assessed by each institution and third-party validation of the data was performed independently by each laboratory (data not shown)

I.V. glucose tolerance test (IVGTT)

After overnight fasting and baseline blood sampling at −15, −10 and −5 min, 0.3 g/kg of 50% glucose was injected over a 1 -min period starting at t = 0. Additional blood samples were collected at t = 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18 and 20, 30, 40 and 60 min after injection. Data from the first 10 min of the test were used to calculate incremental area under the curve (AUC) for insulin (AIR_{GLU}) and C-peptide (ACR_{GLU}) in response to I.V. glucose. AUC was calculated by the trapezoidal rule with the mean of the baseline values subtracted. IVGT was evaluated by glucose disappearance rate [Kg = ln(glucose)/min × 100], calculated as the slope of the natural log of glucose values between 10 and 20 min with least-squares linear regression (7,14).

Arginine stimulation test

The arginine stimulation test (AST) was performed at normoglycemia (~5 mM glucose) after overnight fasting. After baseline blood sampling at −15, −10 and −5 min, 5 g of L-arginine hydrochloride (10% solution; Rgene, Pharmacia Inc., Clayton, NC, USA) was given I.V. over a 30-s period. Blood samples were obtained at t = 2, 3, 4, 5, 7, 10, 15, 20, 25 and 30 min after injection to measure glucose, insulin and C-peptide. Data from t = 2–5 min were used to calculate incremental AUC with the mean of the baseline values subtracted for insulin (AIR_{ARG}) and C-peptide (ACR_{ARG}) by means of the trapezoidal rule (7,13,14).

Glucose-potentiated arginine test

After the AST, GPAIS was performed at hyperglycemia (>15 mM glucose) to calculate maximal acute insulin (AIR_{MAX}) and C-peptide (ACR_{MAX}) responses to arginine. Plasma glucose level was increased over 45 min to >15 mM using a modified hyperglycemic clamp technique with a priming rate of 20% dextrose solution infused over 15 min, subsequently modified based on plasma glucose determinations every 5 min to maintain the hyperglycemic level above 15 mM (15). After 45 min of hyperglycemic clamp, prestimulus blood samples were obtained at −10, −5 and 0min and a second 5 g arginine injection given. Samples and calculations were obtained at the same time intervals using the methodology described above for AST (7,13,14). Slope of glucose potentiation was calculated as previously published (1).

Secretory unit of islet transplant objects (SUITO) index

The SUITO index was calculated at 3, 6 and 12 months post last infusion as previously published (16–18).

Data analysis

AUC and statistical calculations were performed using GraphPad Prism 4 (GraphPad Software, La Jolla, CA, USA). Comparisons between groups were performed by one-way ANOVA and nonparametric ANOVA (Kruskal– Wallis) with *post hoc* testing by Bonferroni's and Dunn's Multiple Comparison Test, respectively. Student's t–test was used to establish comparisons between the two groups. Results are shown as mean±SEM. Significance was established at a p-value <0.05. Receiver operator characteristics′ (ROC) AUC is equal to the probability that a classifier will rank a randomly chosen positive instance higher than a randomly chosen negative one. ROC analysis was implemented to most accurately calculate cutoff values for acute insulin and C-peptide responses derived from each metabolic test. Test cutoff values were selected based upon user-defined balance between the highest level of sensitivity and specificity, which produces the highest likelihood ratio (LR) of accurate discrimination between insulin-independent subjects and those who return to exogenous insulin therapy. An LR value >3 is considered clinically acceptable. For this purpose, all existing values at 3, 6 and 12 months from the three different institutions were used. For each assay, insulin requirement was noted at the time of assessment. The sensitivity, specificity, positive and negative predictive value of each test to predict need for insulin therapy was calculated.

Results

Subject characteristics

Demographics of PIT (n = 30) and their respective controls (n = 10) are summarized in Table 1. Twenty-five subjects achieved insulin independence. Mean follow-up after first islet infusion was almost 5 years (59.9 ± 22 months, range 28–89). Average islet equivalents (IEQ) infused was 16 483 ± 5951 IEQ/kg body weight (BW; Table 2). Donor demographics and islet quality are summarized in Table S1. Five subjects never achieved insulin independence despite initially reduced insulin requirement, improvement in HbA1c and

elimination of hypoglycemic events. Eleven of 24 patients in the ITA group remain insulin independent for >1 year posttransplant. Five of six IAK patients were insulin independent for >1 year. HbA1c decreased from a pretransplant mean of 7.2 ± 1.2% to 5.82 ± 0.8% ($p = 0.002$) 3 months posttransplant and remained significantly lower than pretransplant through 12 months' follow-up (Table 2).

Recovery in glucose disposal rate (Kg) after PIT was observed after I.V. glucose bolus, with values similar to nondiabetic controls. First-phase insulin and C-peptide release after I.V. glucose stimulation are shown in Figure 1. AIR_{GLU} and ACR_{GLU} were significantly decreased (threefold lower) in islet recipients versus controls at 3, 6 and 12 months posttransplantation ($p < 0.001$; Table 3, Section A).

Figure 2 demonstrates insulin and C-peptide responses to arginine under normoglycemic conditions. AIR_{ARG} was approximately half in islet recipients versus controls at 3 months ($p = 0.02$), but not at 6 and 12 months (NS; Table 2, Section B). Similarly, ACR_{ARG} was significantly lower (twofold) in the transplant group versus controls at 3 months ($p = 0.002$) and at 6 and 12 months ($p < 0.05$; Table 3, Section B).

GPAIS is demonstrated in Figure 3. AIR_{MAX} and ACR_{MAX} were significantly decreased (threefold lower) in islet recipients versus controls at 3, 6 and 12 months posttransplantation ($p < 0.0001$; Table 3, Section C).

Slope of potentiation, an index of maximal β-cell secretory reserve (Figure 3, panel B), also demonstrated a reduced reserve in PIT compared to controls. At 3, 6 and 12 months, slope of potentiation is significantly lower in PIT (125.1 ± 36.3, 158 ± 46.3 and 93.2 ± 23.5) compared to Non-diabetic control group (NDC) (574.9 ± 142; $p < 0.001$, 0.01 and 0.001, respectively).

The SUITO index at 3, 6 and 12 months post last infusion (Table 3, Section D) is also reduced in islet recipients versus controls.

Islets transplanted as a predictor of insulin independence 1 year post last infusion

The number of IEQs per kilogram BW was compared between patients remaining insulin independent (16 515 ± 5229 IEQ/kg vs. 18 654 ± 6724 IEQ/kg) and those insulin dependent 1 year after last islet infusion ($p= 0.345$). No correlation was established between IEQ/kg BW and changes in HbA1c% at 3 months post last infusion ($r^2 = 0.01$, $p = 0.53$). In addition, no correlation was established between IEQ/kg BW transplanted and units of exogenous insulin/kg BW required after the first year post last islet infusion ($r^2 = 0.01$, $p = 0.53$; Figure 4).

We then established whether a correlation might exist between IEQ/kg BW transplanted and functional islet mass engrafted. Insulin and C-peptide secretion at 3 months post last infusion were used as surrogate markers of β-cell mass engrafted. No correlation was identified between AIR_{GLU}, ACR_{GLU}, AIR_{ARG}, ACR_{ARG}, AIR_{MAX} and IEQ/kg BW transplanted(Figure 5). However, only ACR_{MAX} and IEQ/kg BW of transplanted islets had a modest but significant correlation ($r^2 = 0.48$, $p = 0.049$).

Establishment of cutoff values for acute insulin and C-peptide response for IVGTT, AST at normoglycemia and hyperglycemia levels corresponding to exogenous insulin use requirement

Figure 6 summarizes AIR_{GLU} (panel A), AIR_{ARG} (panel B) and AIR_{MAX} (panel C) for all subjects. Patients were stratified in two groups (off vs. on) according to insulin requirement at time of metabolic assay and compared to nondiabetic controls. AIR_{GLU} was greater in patients remaining insulin independent versus those returning to insulin after PIT (1143 ± 113.2 vs. 81.6 ± 67.5 pmol/L min, $p < 0.001$). AIR_{ARG} values were no different in insulin-independent patients (599.8 ± 51.2 pmol/L min) compared to controls (708 ± 260 pmol/L min, $p > 0.05$). No differences were noted between patients on and off insulin after PIT (423.3 ± 53.4 pmol/L min, $p > 0.05$; panel B). AIR_{MAX} was greater in the insulin-independent versus insulin-dependent groups (1623 ± 285 vs. 836 ± 100 pmol/L min), but significantly lower in both PIT groups compared to controls (6817 ± 1527 pmol/L min, $p < 0.01$ and $p < 0.001$, respectively).

Figure 6 (panels D–F) represents ROC analysis used to calculate cutoff values for insulin in each metabolic test. The AUC for ROC analysis was similar between AIR_{GLU} (0.93, $p = 0.0001$) and AIR_{MAX} (0.95, $p = 0.001$); however, AIR_{GLU} showed the best power of discrimination, with a LR of (6.27–) for a cutoff of 356 pmol/L min. This cutoff had a sensitivity of 88% a specificity of 85.9% with a positive and negative predictive value 93% and 78%, respectively. Very similar to AIR_{GLU}, the AIR_{MAX} also has an excellent clinically acceptable LR of 6.0, for a cutoff value of 1509 pmol/L min, with sensitivity and specificity of 100% and 83%, respectively, and positive and negative predictive value for both of 100% ($n = 27$).

In contrast, AIR_{ARG} has a clinically unacceptable LR (2.28), with a moderate sensitivity of 66.6% and specificity of 70.7%, a reasonable positive predictive value of 72.7%, but an unacceptable negative predictive value of 33%.

Figure 7 summarizes acute C-peptide response to I.V. glucose (panel A), arginine (panel B) and GPAIS (panel C). ACR_{GLU} was the only test capable of discriminating differences between PIT recipients off and on exogenous insulin (2.67 ± 0.22 vs. 0.32 ± 0.2 nmol/L min, $p < 0.001$). A statistically greater AUC was seen in the nondiabetic control group (7.28 ± 1.22 nmol/L mi) compared to PIT recipients on or off exogenous insulin ($p < 0.001$ for both). In contrast, ACR_{ARG} and ACR_{MAX} are only capable of differentiating between islet recipients and their controls ($p < 0.001$ for both), but not between insulin-independent patients versus patients returning to exogenous insulin after PIT (panels B and C).

Figure 7 (panels D–F) represents ROC analysis to calculate the cutoff value for each test. The ACR_{GLU} ROC has an AUC of 0.92 ($p < 0.0001$). For an ACR_{GLU} cutoff value of 0.87, this test has the highest sensitivity and specificity (89% and 83%, respectively) and the best LR (5.37), with a positive and negative predictive value of 93% and 75%, respectively. Neither ACR_{ARG} nor ACR_{MAX} provides a useful cutoff value differentiating patients requiring exogenous insulin therapy compared to those who do not. The positive and negative predictive value for each test was calculated based on data available at 12 months and summarized at the bottom of Figure 7.

Is lower islet β -cell mass engraftment at 3 months post-PIT associated with return to insulin dependence by 12 months?

When PIT recipients were separated into two groups (insulin independent 1 year posttransplant vs. partial β-cell function as determined by detectable fasting C-peptide but requiring exogenous insulin at 1 year), a difference in AIR_{GLU} at 3 months was observed between the two subgroups (Figure 8, upper panel).

In contrast, no significant differences at 3 months were seen when AIR_{ARG}, ACR_{ARG}, AIR_{MAX} and ACR_{MAX} results were compared between on and off insulin at 1 year (Figure 8, middle panel).

Discussion

Clinical trials have shown that insulin independence can be consistently achieved when a sufficient number of islets is implanted (>10 000 IEQ/kg recipient BW; Ref. 19). However, the correlation between islet mass transplanted and engraftment and the potential to predict the stage of insulin independence beyond 1 year after transplantation based on number of transplanted islets remains unknown. Transplanting the largest possible number of islets is considered among the most important factors for success, but other factors, including quality of transplanted islets (20), instant blood-mediated inflammatory response (21) and inefficient neovascularization of the graft (22), account for the lack of correlation between transplanted islet mass and engraftment. Interestingly, our data shows that transplanted mass (IEQ/kg BW) is an unreliable predictor of insulin independence and correlates poorly with function at 1 year post-transplant.

Diminished first-phase insulin response to I.V. glucose is recognized as an early marker of β-cell dysfunction, appearing before significant impairment in glucose tolerance. Our PIT recipients showed a decreased first-phase insulin release, paralleling findings in other populations at increased risk for overt diabetes development (23,24). Sufficient functional islet β-cell mass is necessary for restoration of first-phase insulin release which is not accomplished after PIT using an “Edmonton-like” immunosuppressive protocol. In addition, we have also demonstrated that AIR_{ARG} is neither sensitive nor specific enough to unmask differences in functional β-cell mass between islet recipients remaining insulin independent and those returning to insulin, as previously demonstrated in streptozotocin-induced β-cell loss in nonhuman primates (25,26). Similar findings of preserved AIR_{ARG} when the response to AIR_{GLU} is minimal or absent have also been reported by Rickels et al. in PIT (3), early T1D (10), type 2 diabetes (11,27) and in partially functioning solid organ pancreas transplants (2). Our results are also analogous to previous findings in which β-cell response to GPAIS correlated best with directly measured β-cell mass (26,28), as AIR_{MAX} provided the greatest discrimination in functional β-cell mass between islet recipients and nondiabetic controls and the highest sensitivity and specificity to discriminate between patients that are insulin independent and those who return to insulin therapy.

We sought to provide cutoff values for each individual metabolic test paralleling its accuracy in predicting insulin dependence. Based on ROC analysis, our results clearly indicate AIR_{GLU} and AIR_{MAX} provide similar AUC. Similarly, the LR to discriminate

between the insulin-independent and insulin-dependent subgroups are alike for AIR_{GLU} LR of 6.27, for a cutoff value of 356 pmol/L min, compared to an LR of 6 for a cutoff value of 1509 pmol/L min for the AIR_{MAX}. Both sensitivity and specificity are within acceptable range for clinical use, with excellent positive and negative predictive values. In contrast, AIR_{ARG} is not an acceptable method to follow clinical islet transplantation.

For C-peptide measurement, ACR_{GLU} provides the only clinically acceptable method to differentiate between insulin-independent recipients versus those who return to insulin (AUC = 0.92, p = 0.0001) with a cutoff value between 0.87 nmol/L min, representing LR of 5.37. The cutoff values established for ACR_{ARG} and ACR_{MAX} did not sufficiently discriminate between patients on and off insulin.

The rationale to determine cutoff values was intended to provide a standardized tool to guide clinicians on the significance/use of their results, specifically for PIT recipients. If this cutoff value was to be used prospectively, it could provide guidance in the following areas: (1) to define the best time for retransplantation; (2) to initiate potential therapeutic interventions aimed at preserving or increasing islet β-cell mass and/or function and (3) to define a surrogate end point for future clinical trials in which the benefit of a therapeutic intervention may be measured with short-term follow-up. Clearly, usage of these values must be rigorously tested with a larger database and with different immunosuppressive protocols.

Our results also demonstrate that AIR_{GLU} is of great value in discriminating changes in functional β-cell mass over time. Based on AIR_{GLU}, we determined that exogenous insulin dependence 12 months postPIT was associated with lower islet mass engraftment 3 months after islet infusion (Figure 8). In addition, a positive correlation was observed between islet mass engraftment-detected AIR_{GLU} and days of insulin independency (Figure 9).

We have also extended our analysis to compare IVGTT to previously simplified indexes that have proven to correlate with insulin independence (16). Specifically, we have observed that the SUITO index calculated at 3 months post islet transplantation correlated well with AUC for blood glucose, AIR_{GLU} and ACR_{GLU} after IVGTT changes posttransplant and for HbA1c changes at 12 months after last infusion. However, the SUITO index calculated at 3 months failed to predict patients who required exogenous insulin therapy at 12 months. Another important finding in our analysis is that the average SUITO index at 3, 6 and 12 months in patients who were insulin dependent were significantly greater than a SUITO index of 26, the established cutoff proposed as a discriminator between insulin-independent and insulin-dependent patients (Figures S1 and S2).

Study limitations are based on the restricted number of patients available to establish the predictive model described here, the lack of an alternative population of islet transplant recipients to validate the results, the short-term metabolic follow-up (12 months) and the inability to compare our data with other simplified beta scores published previously (29). Despite these limitations, this manuscript represents the largest series of sequential metabolic testing evaluating β-cell secretory capacity as a surrogate marker of functional β-cell mass.

In conclusion, these data support that AIR_{GLU} and AIR_{MAX} were estimated to be the two best methods to determine insufficient islet mass engraftment associated with return to insulin dependence within 1 year after PIT. In addition, our findings demonstrate that AIR_{GLU} is the best method to serve as an early metabolic marker anticipating loss of insulin independence in T1D islet allograft recipients. In light of the observed results, wide availability and simple methodology, we strongly support the usage of AIR_{GLU} as the optimal method to follow patients after islet transplantation.

While acknowledging that the loss of islet mass over time is likely multifactorial, return to insulin dependence in some islet recipients may be due to impaired β-cell secretory capacity related to insufficient initially engrafted β-cell mass, leading to progressive β-cell functional deterioration.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

ACR_{ARG}	acute C-peptide response to arginine
ACR_{MAX}	acute C-peptide response to glucose-potentiated arginine-induced insulin secretion
ACR_{GLU}	acute C-peptide response to glucose
Kg	glucose disappearance rate
AIR_{ARG}	acute insulin response to arginine
AIR_{GLU}	acute insulin response to glucose
AIR_{MAX}	acute insulin response to glucose-potentiated arginine-induced insulin secretion
AST	arginine stimulation test

AUC	area under the curve
DBD	donation after brain death
DCD	donation after cardiocirculatory death
GPAIS	glucose-potentiated arginine-induced insulin secretion
IAK	islet after kidney
ITA	islet transplant alone
IVGTT	I.V. glucose tolerance test
K	nephropathy
LR	likelihood ratio
N	neuropathy
PIT	pancreatic islet transplantation
R	retinopathy
ROC	receiver operator characteristics
SUITO	secretory unit of islet transplant objects
T1D	type 1 diabetes
UW	University of Wisconsin

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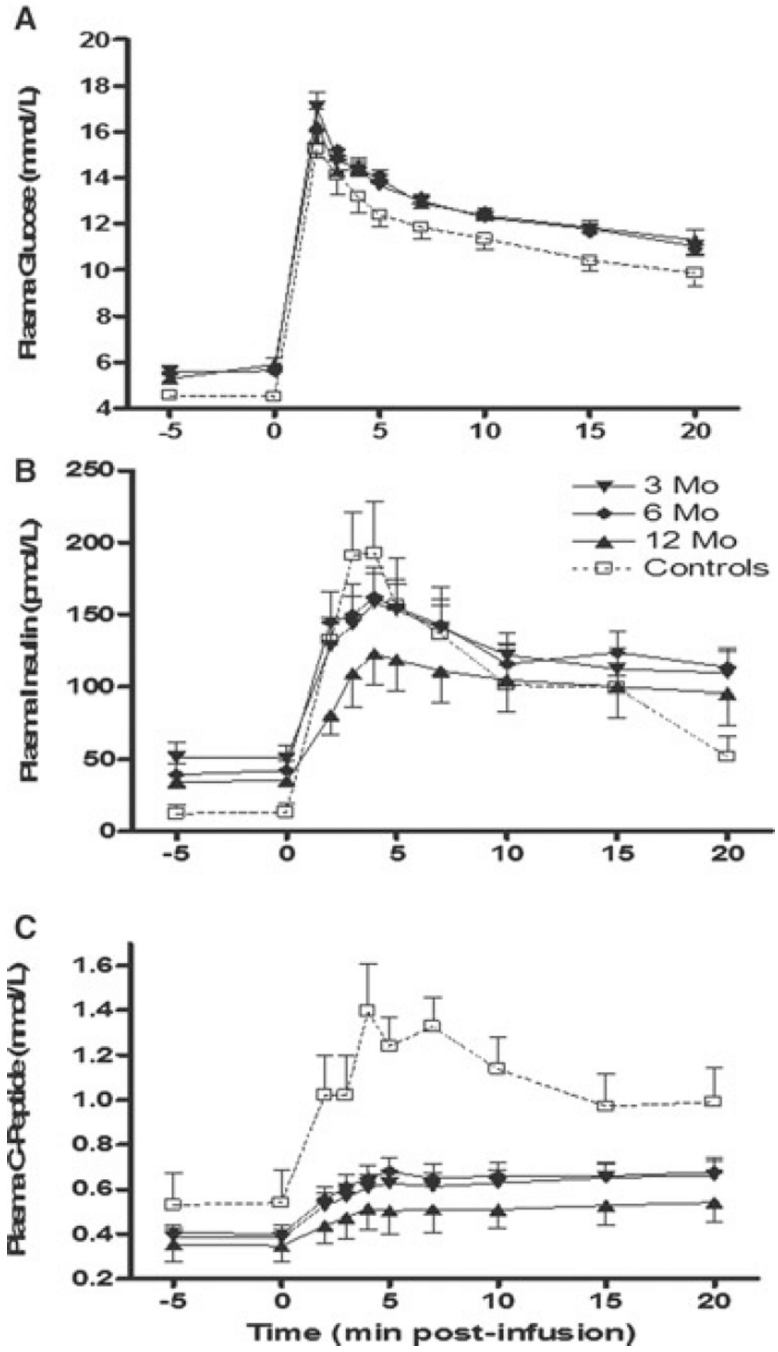


Figure 1. Intravenous glucose tolerance test in islet transplant recipients at 3, 6 and 12 months posttransplant

(A) Glucose kinetics over 20 min after 300 mg/kg dextrose administration over 1 min starting at t = 0. (B) Levels of insulin and (C) C-peptide release were plotted against nondiabetic control subjects. Thirty patients were assessed at 3 months, 19 patients were assessed at 6 months and 27 patients were assessed at 12 months post last infusion. Data are expressed as mean ± SE.

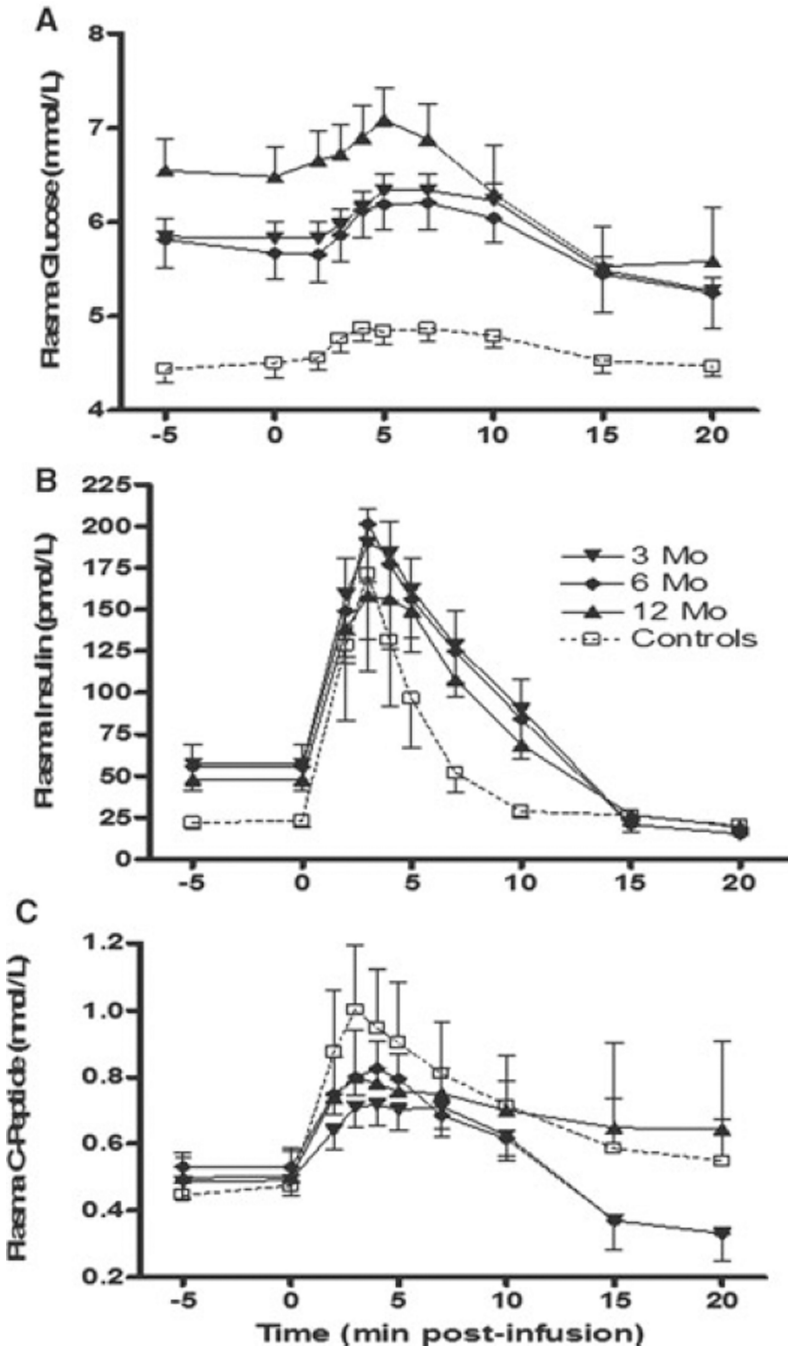


Figure 2. Arginine stimulation test in islet transplant recipients at 3, 6 and 12 months posttransplant

(A) Glucose kinetics over 20 min after 5 g arginine administration over 30 s starting at t = 0. (B) Levels of insulin and (C) C-peptide release were plotted against nondiabetic control subjects. Thirty patients were assessed at 3 months, 19 patients were assessed at 6 months and 27 patients were assessed at 12 months post last infusion. Data are expressed as mean ± SE.

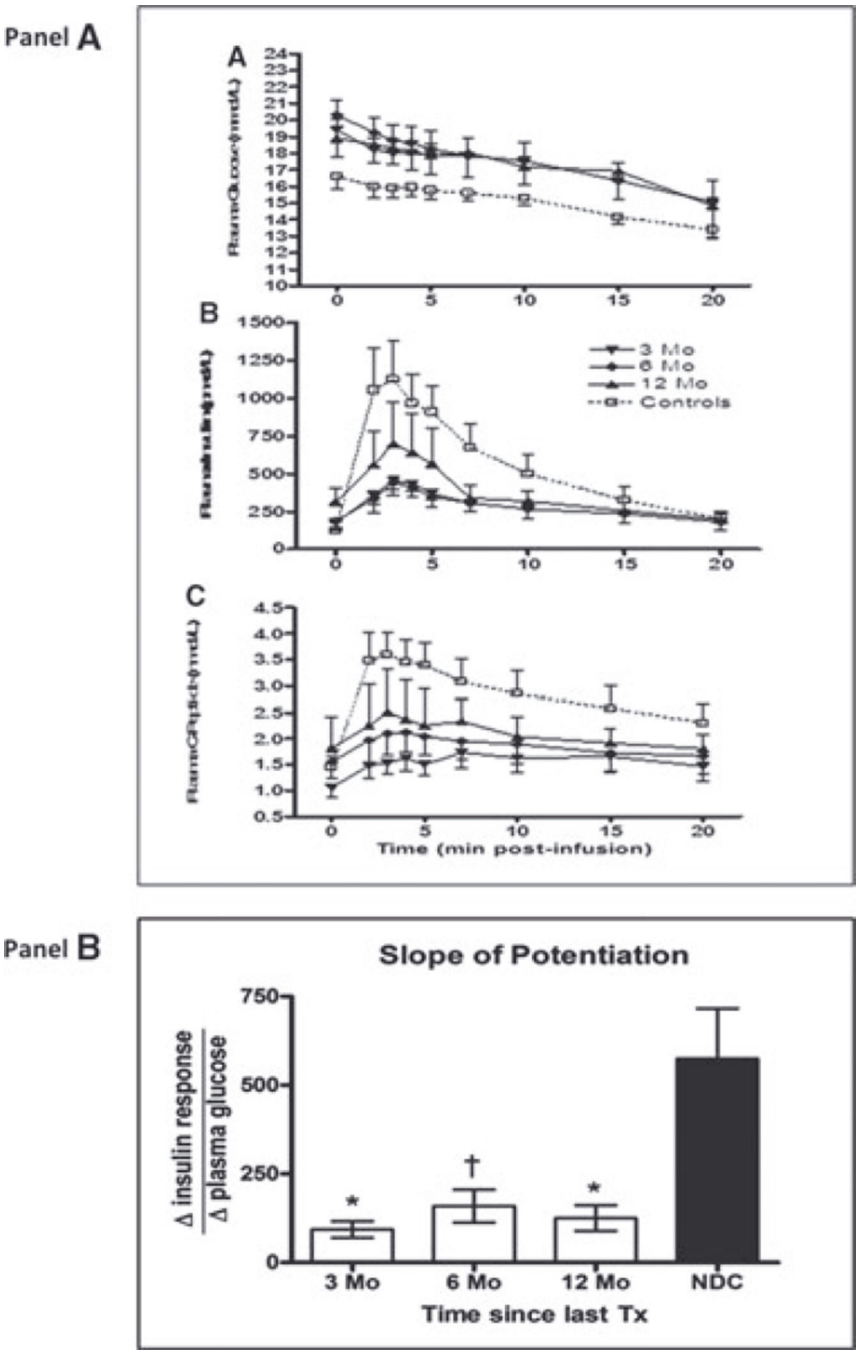


Figure 3. Panel (A): Glucose potentiation of arginine induced insulin secretion (GPAIS) in islet transplant recipients at 3, 6 and 12 months posttransplant

Thirteen patients were assessed at 3 months, 8 patients were assessed at 6 months and 13 patients were assessed at 12 months post last infusion. (A) Glucose kinetics over 20 min after 5 g arginine administration over 30 s starting at t = 0. (B) Levels of insulin and (C) C-peptide release were plotted against nondiabetic control subjects. Data are expressed as mean ± SE. Panel (B): Slope of glucose-potentiation is calculated as the change in insulin release in response to arginine from the normoglycemic to the hyperglycemic condition, divided by the change in plasma glucose. The posttransplant calculated slope in PIT

recipients was 1.2 ± 0.4 (p = 0.02) at 3 months, 1.2 ± 0.5 (p = 0.02) at 6 months, 1.6 ± 1.1 (p = 0.07) at 12 months and 1.8 ± 1.0 (p = 0.08) at 24 months, compared to a slope of 5.1 ± 1.4 for controls.

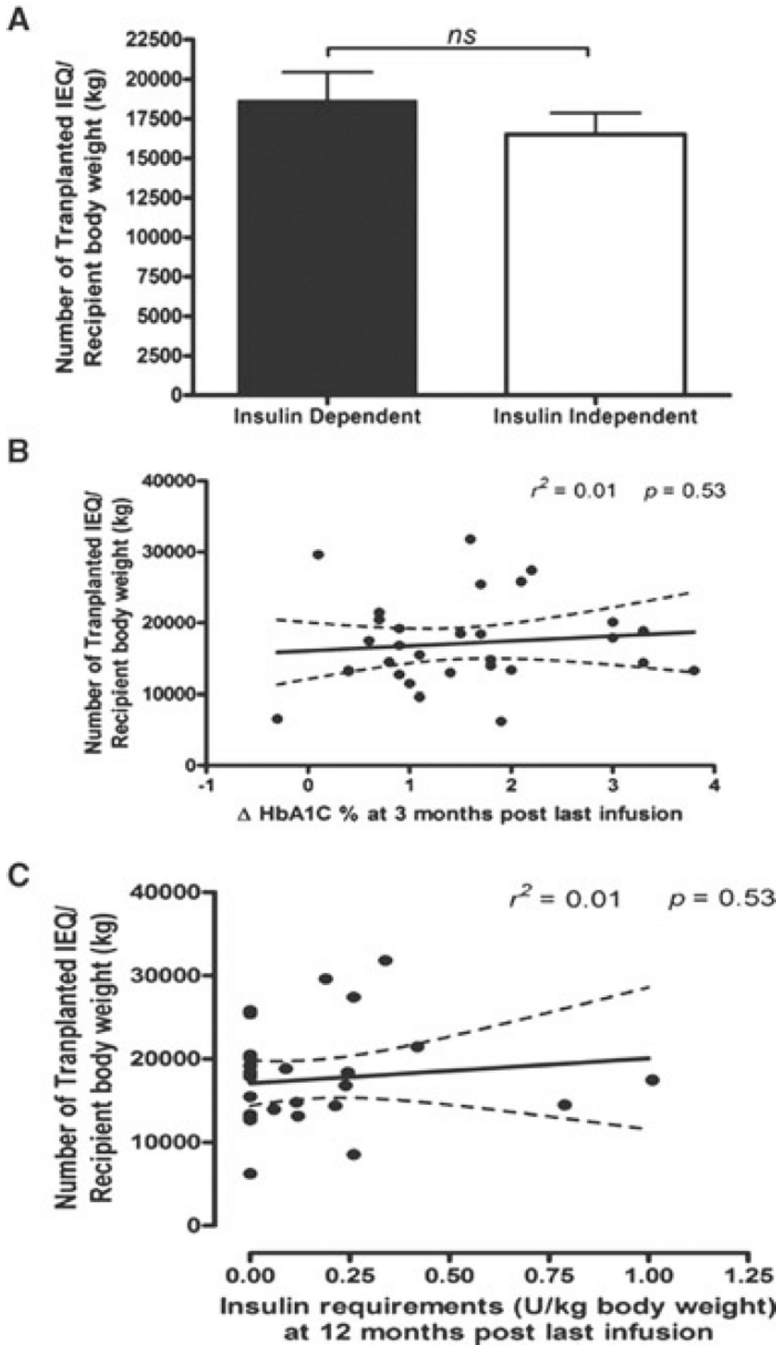


Figure 4. Comparison of total transplanted islet mass and metabolic assessment in the posttransplant period

Panel (A): Bar representation of transplanted islet mass and insulin requirement status at 12 months post last islet infusion. Insulin-dependent group (n = 14) and insulin-independent group (n = 15). Mean and SEM were calculated in each group. Statistical significance was considered at $p < 0.05$. Panel (B): Relation of total transplanted islet mass post last infusion with insulin secretion AUC at 12 months post last infusion (n = 29). Statistical significance was considered at $p < 0.05$. Panel (C): Relation of total transplanted islet mass post last

infusion with HbA1C level at 3 months post last infusion normalized to pretransplant level (n = 29). Statistical significance was considered at $p < 0.05$.

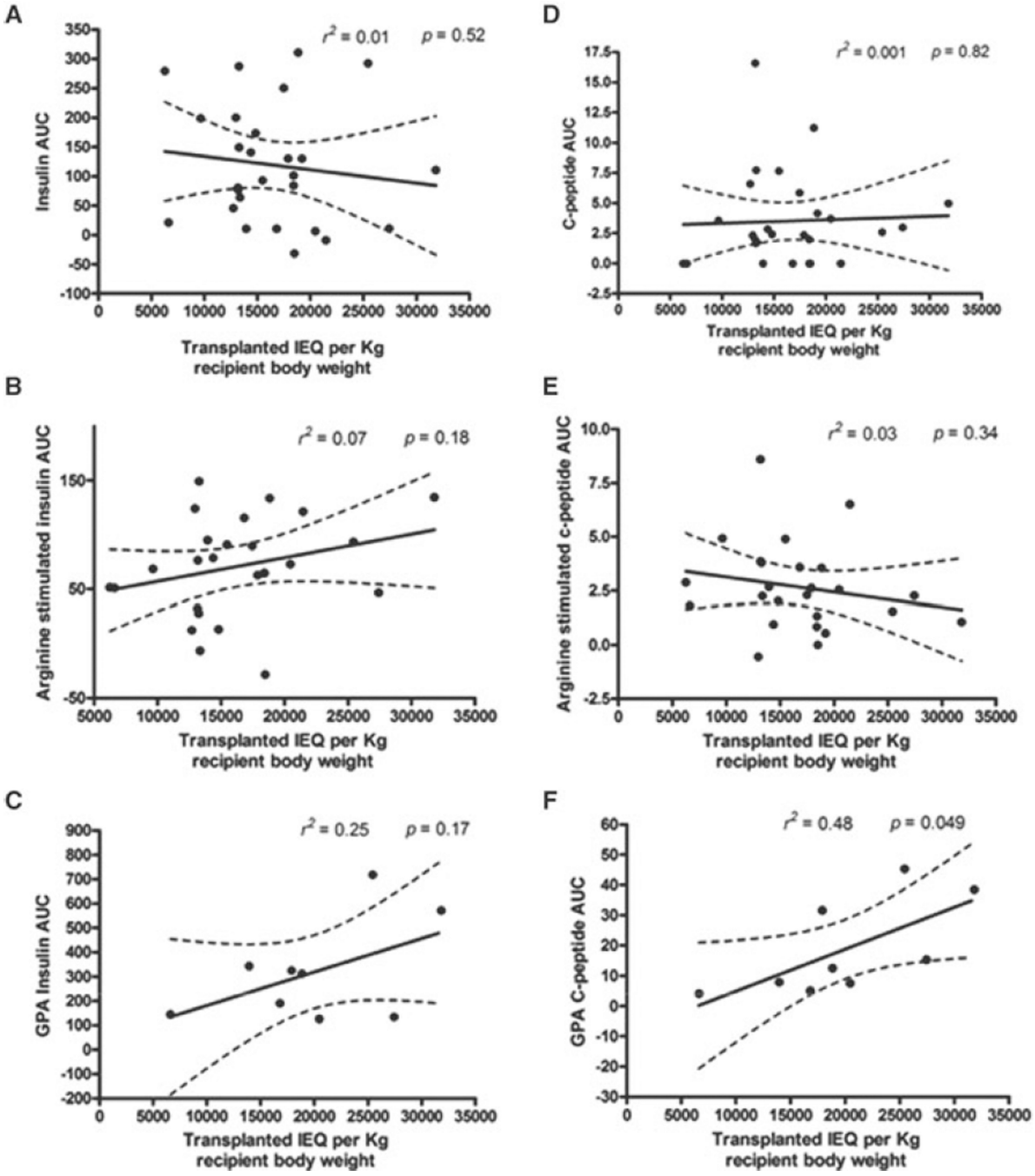
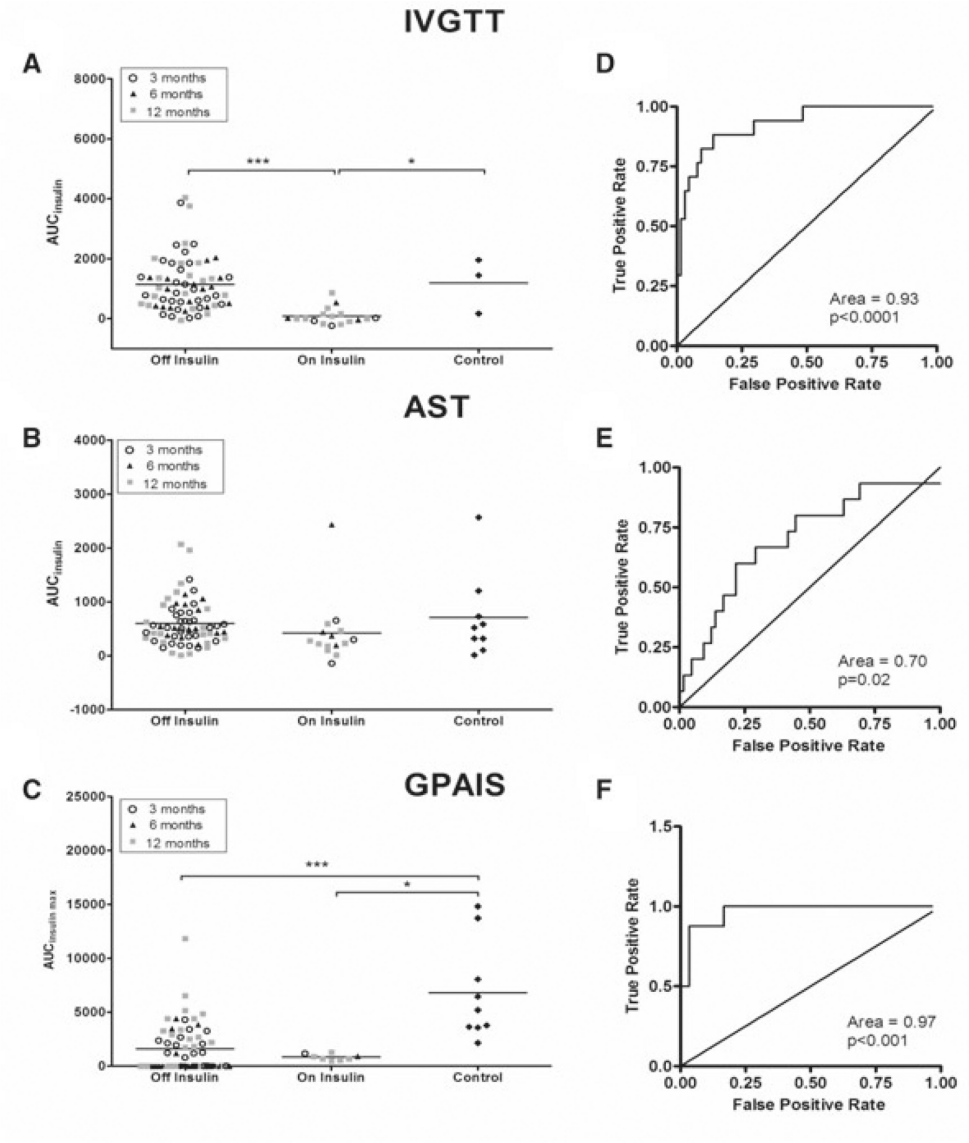


Figure 5. Comparison of total transplanted islet mass and insulin and C-peptide levels after IVGTT, arginine stimulation test and glucose-potentiated arginine test

Panel (A): Relation of total transplanted islet mass post last infusion with insulin secretion AUC after IVGTT at 3 months post last infusion (n = 27). Statistical significance was considered at $p < 0.05$. Panel (B): Relation of total transplanted islet mass post last infusion with insulin secretion AUC after arginine stimulation test at 3 months post last infusion (n = 25). Statistical significance was considered at $p < 0.05$. Panel (C): Relation of total transplanted islet mass post last infusion with insulin secretion AUC after glucose-

potentiated arginine test at 3 months post last infusion (*n* = *9*). Statistical significance was considered at $p < 0.05$. Panel (D): Relation of total transplanted islet mass post last infusion with c-peptide secretion AUC after IVGTT at 3 months post last infusion (n = 27). Statistical significance was considered at $p < 0.05$. Panel (E): Relation of total transplanted islet mass post last infusion with c-peptide secretion AUC after arginine stimulation test at 3 months post last infusion (n = 25). Statistical significance was considered at $p < 0.05$. Panel (F): Relation of total transplanted islet mass post last infusion with c-peptide secretion AUC after glucose-potentiated arginine test at 3 months post last infusion (n = 9). Statistical significance was considered at $p < 0.05$.

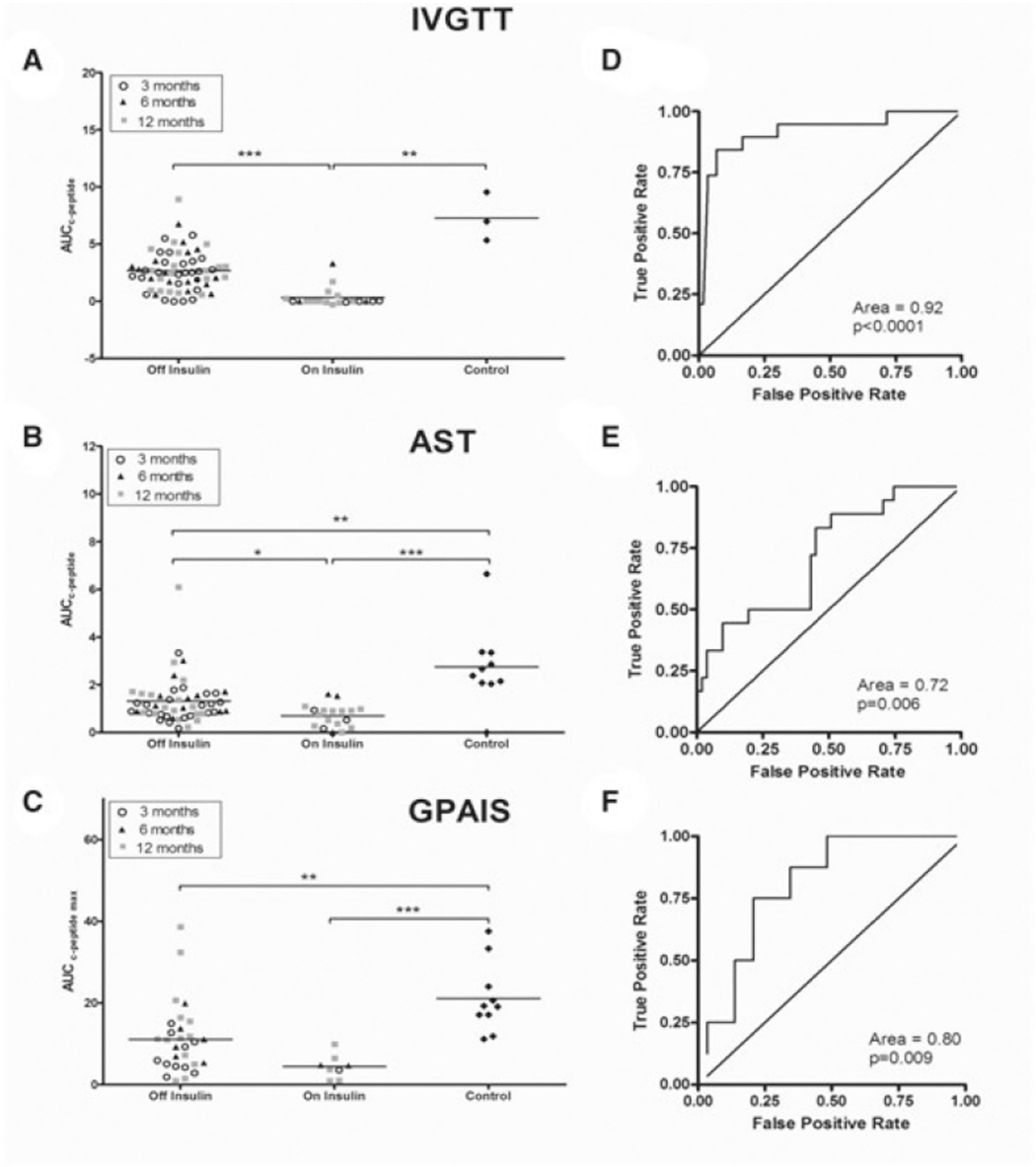


	Cut-off Values AUC	Sensitivity (%)	Specificity (%)	LR	PPV (%)	NPV (%)
IVGTT	355.5	88.2	85.9	6.27	92.8	77.7
AST	374.5	66.6	70.7	2.28	72.7	33.3
GPAIS	1509	100	83.3	6.0	100	100

Figure 6. Distribution plots for Insulin secretion using the ROC curve and analysis for IVGTT, arginine stimulation test and GPAIS

Panels (A)–(C): All sequential AIR_{GLU}, AIR_{ARG} and AIR_{MAX} for UW patients during 24-month follow-up. Acute insulin response data were segregated according to exogenous insulin dependence. Mean and SEM were calculated in each group. Data are also stratified according to whether the test was performed 3, 6 or 12 months after the last islet transplantation. Statistically significant differences are expressed as *($p < 0.05$), **($p < 0.01$) and ***($p < 0.001$). Panels (D)–(F) represent the receiver operator characteristic for AIR_{GLU}, AIR_{ARG} and AIR_{MAX}, respectively. It was determined at the time of each assay

whether the patient required insulin to achieve normoglycemia and recorded as “On Insulin” or “Off Insulin.” The ROC graph recorded a point for each data pair (quantitative result, clinical outcome) as if it was the critical value for a predictive assay and considering the data set at that point as true positives and false positives. All data from sequential measurements at 3, 6 and 12 months post last islet infusion was included. Area under the ROC curve was then calculated. Tests which cannot discriminate between true and false positives show a sensitivity plot that is not significantly different from the line of identity and a p-value >0.05 when the AUC is calculated. Cutoff values that generate the highest sensitivity and specificity using the best likelihood ratios were chosen for each assay.



	Cut-off Values *AUC*	Sensitivity (%)	Specificity (%)	LR	PPV (%)	NPV (%)
IVGTT	0.87	89.4	83.3	5.37	92.8	75.0
AST	1.012	83.3	54.9	1.9	90.0	53.8
GPAIS	6.7	87.5	65.5	2.5	100	66.6

Figure 7. Distribution plots for C-peptide secretion using the ROC curve and analysis for IVGTT, arginine stimulation test and GPAIS

Panels (A)–(C): All sequential ACR_{GLU}, ACR_{ARG} and ACR_{MAX} for UW patients during 24-month follow-up. Acute C-peptide response data was segregated according to their exogenous insulin dependency. Mean and SEM were calculated in each group. Data is also stratified according to whether the test was performed 3, 6 or 12 months after the last islet transplantation. Statistically significant differences were expressed as *($p < 0.05$), **($p < 0.01$) and ***($p < 0.001$). Panels (D)–(F) represent the receiver operator characteristics for ACR_{GLU}, ACR_{ARG} and ACR_{MAX}, respectively. Tests, which cannot discriminate between

true and false positives, show a sensitivity plot that is not significantly different from the line of identity and a p-value >0.05 when the AUC is calculated. All data from sequential measurements at 3, 6 and 12 months post last islet infusion was used. Area under the ROC curve was then calculated. Cutoff values that generated the highest sensitivity and specificity using the best likelihood ratios were chosen for each assay.

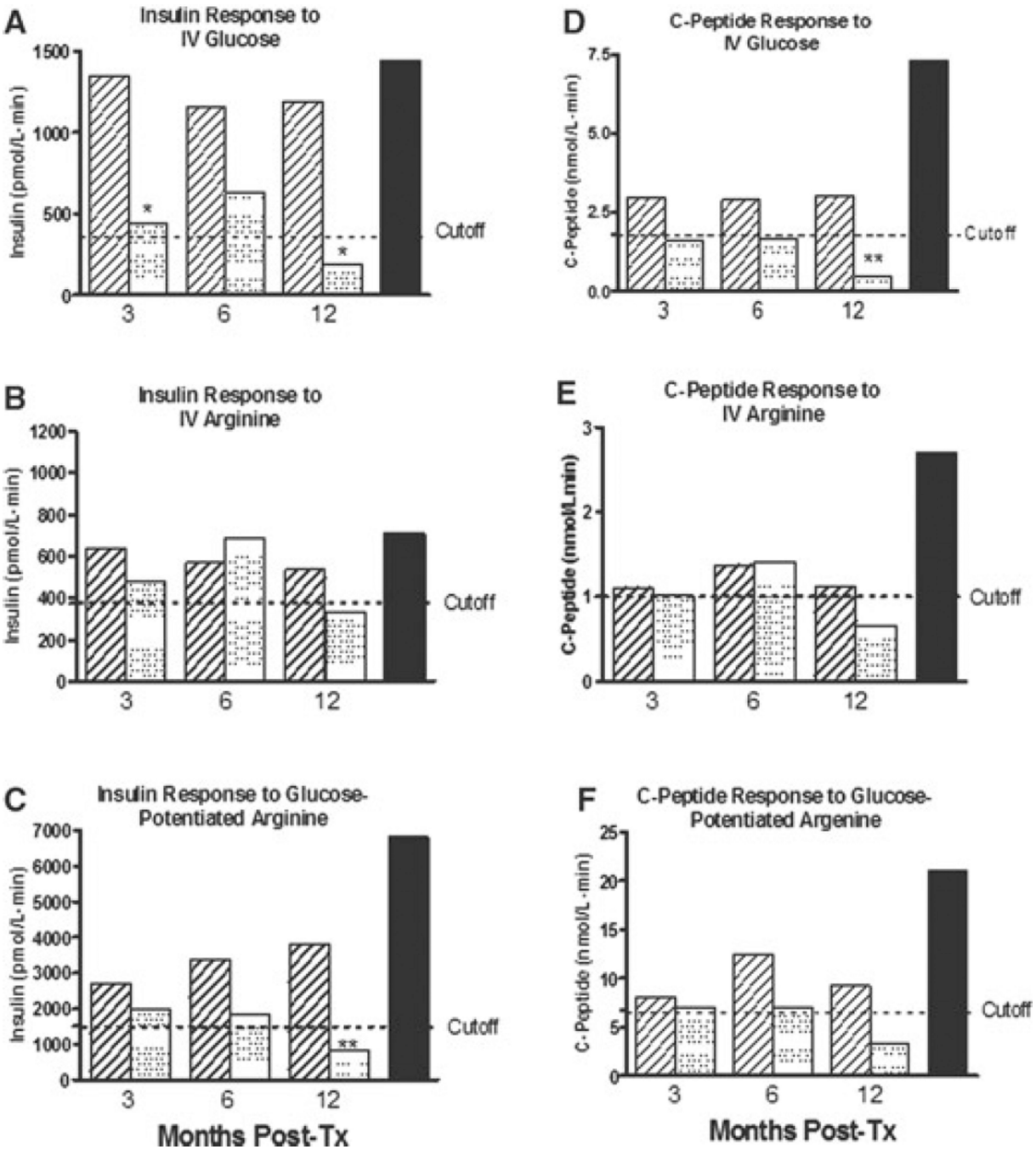


Figure 8. Comparison between acute insulin and peptide response in 32 islet transplant recipients from three institutions

Patients were divided according to their exogenous insulin requirement 12 months posttransplant. Acute insulin and C-peptide response is expressed as mean ± SEM. Cutoff values from Figures 6 and 7 are overlapped with the bars, representative of the data. Statistically significant differences were expressed as *($p < 0.05$) and **($p < 0.01$). Nondiabetic controls are represented for comparison. Panels (A) and (B): Bar representation of the AIR_{GLU} and ACR_{GLU} response at 3, 6 and 12 months after last transplant. Panels (C) and (D): Bar representation of the AIR_{ARG} and ACR_{ARG} response at 3, 6 and 12 months

after last transplant. Panels (E) and (F): Bar representation of the AIR_{MAX} and ACR_{MAX} response at 3, 6 and 12 months after last transplant.

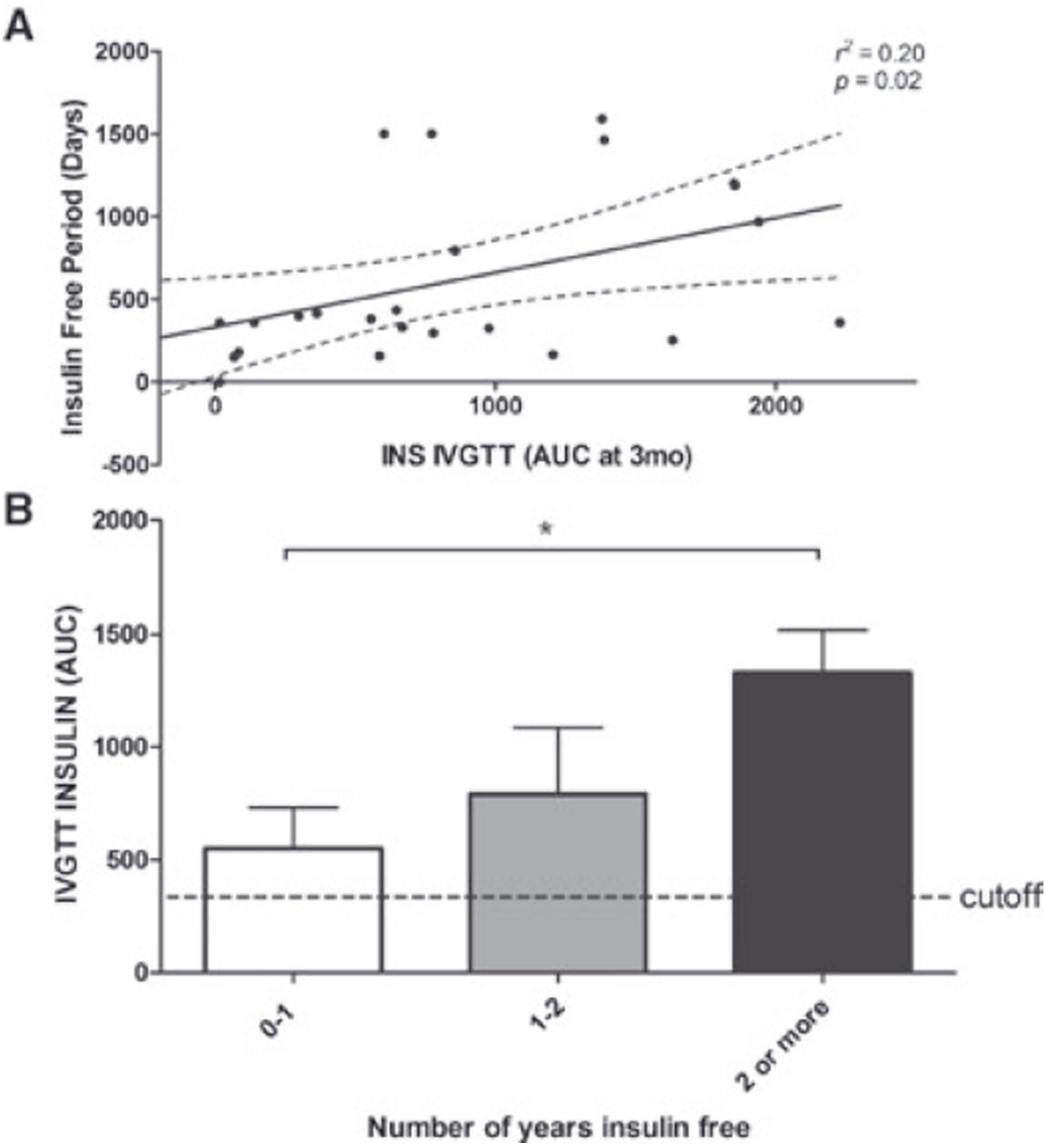


Figure 9. Insulin IVGTT performed at 3 months as a predictor of posttransplant insulin independence and long-term graft function

Panel (A): Correlation between IVGTT acute insulin response at 3 months and posttransplant insulin independence. Panel (B): Comparison between IVGTT insulin AUC performed at 3 months and long-term insulin independence. Data are mean ± SEM. Statistical significant differences expressed as *($p < 0.05$), **($p < 0.01$) and ***($p < 0.001$).

Table 1

Baseline demographic data for all islet transplant recipients (by center)

Patients	Age (years)	Gender (M/F)	Weight (kg)	BMI (kg/m^2)	Diabetes (years)	Insulin requirement pre-Tx (units/kg/day)	Complications related to T1DM	HbA1c pre-Tx (%)	Hypoglycemia unawareness	Creatinine clearance (mL/min)
Center 1										
1	32	M	75.5	26.2	25	0.45	R	7.8	Yes	88.6
2	45	M	67.9	22.0	29	0.52	R	4.8	Yes	112
3	54	M	61.8	19.5	38	0.32	None	6.2	Yes	105.5
4	28	M	78.6	26.5	9	0.55	None	7.4	Yes	131.6
5	39	F	47.6	17.6	36	0.46	R, K	9.8	Yes	63.1
6	42	F	57.9	23.2	33	0.45	R, K	7.7	Yes	75.7
Center 2										
7	53	M	63	21.5	41	0.42	R, N	6.1	Yes	102
8	48	F	73	23.8	36	0.47	None	8.6	Yes	87
9	44	M	62	23.1	35	0.64	R, N, K	6.3	Yes	53
10	45	F	76	27.1	33	0.62	R, N	9.0	Yes	111
11	41	F	75	21.5	29	0.65	N	6.1	Yes	112
12	25	M	77	22	11	0.65	None	7.4	Yes	134
13	31	M	77	25.3	28	0.87	None	7.7	Yes	124
14	61	F	53	22	36	0.5	R, N, K	7.8	Yes	78
15	51	F	60	25.8	36	0.4	R, N, K	7.5	Yes	55
16	43	F	56	21.1	41	0.57	R, N, K	6.7	Yes	52
Center 3										
17	37	M	79	23.5	10	0.5	None	7.5	Yes	142
18	41	F	66	23.5	31	0.5	R	7.2	Yes	96
19	32	F	68	25.7	19	0.47	R	9.4	Yes	128
20	38	M	74	24.7	14	0.47	None	7.3	Yes	124
21	43	F	57	23.7	37	0.54	R	7.7	Yes	119
22	44	F	70	25.7	30	0.56	R, K	9.4	Yes	82
23	36	M	69	26.6	34	0.58	R	8.0	Yes	129
24	35	M	97	28	18	0.33	R	8.3	Yes	103

Patients	Age (years)	Gender (M/F)	Weight (kg)	BMI (kg/m^2)	Diabetes (years)	Insulin requirement pre-Tx (units/kg/day)	Complications related to T1DM	HbA1c pre-Tx (%)	Hypoglycemia unawareness	Creatinine clearance (mL/min)
25	36	M	66	26	34	0.53	R	6.8	Yes	102
26	51	M	88	26	39	0.48	None	7.0	Yes	173
27	59	M	86	27	46	0.57	R	8.3	Yes	88
28	52	F	53	22	44	0.29	R	7.6	Yes	81
29	56	F	59	23	35	0.32	None		Yes	75
30	40	F	60	23	12	0.55	None	7.1	Yes	125

Table 2

Individual transplant characteristics and posttransplant hemoglobin A1C (HbA1c) level and insulin requirements for all patient (by center)

Patient	Type of Tx	Number of infusions	Number of IEQ Tx/kg	Pre-Tx HbA1c	3 m HbA1c%	6 m HbA1c%	12 m HbA1c%	12 m insulin
Center 1								
1	ITA	2	15 275	7.8	6.9	7.3	9.5	0.24
2	ITA	2	8541	4.8	6.0	6.7	6.4	0.26
3	ITA	1	6602	6.2	6.5	6.9	7.5	0
4	ITA	4	18 473	7.4	5.9	6.5	6.7	0.18
5	IAK	2	20 085	9.8	6.8	6.0	5.9	0
6	IAK	2	16 530	7.7	4.4	5.8	6.1	0.09
Center 2								
7	ITA	3	20 466	6%	5.3	6.7	6.7	0
8	ITA	2	11 440	8.9	7.9	*	*	*
9	IAK	3	17 886	7.5	4.5	<4	4.8	0
10	ITA	2	31 810	7.4	5.8	6.8	6.9	0.34
11	IAK	2	25 430	7.7	6	6.1	5.3	0
12	ITA	2	25 817	7.3	5.2	5.6	5.6	0
13	ITA	3	29 614	7.1	7	7.4	8.4	0.19
14	ITA	3	27 419	8	5.8	5.6	6.3	0.26
15	IAK	2	19 187	6.7	5.8	6	5.9	0
16	IAK	2	18 397	7.5	5.8	6.1	5.6	0
Center 3								
17	ITA	3	17 467	6.7	6.1	6.3	5.7	1.01
18	ITA	2	13 154	6.2	5.8	5.0	7.6	0.12
19	ITA	4	13 252	9.4	5.6	5.4	5.9	0
20	ITA	2	12 956	7.3	5.9	5.6	5.5	0
21	ITA	2	18 402	7.1	5.4	5.8	5.8	0.25
22	ITA	2	14 383	9.3	6.0	5.8	6.4	0.21
23	ITA	2	13 354	8.0	6.0	6.4	5.9	0
24	ITA	2	6250	7.4	5.5	5.6	5.6	0
25	ITA	3	15 482	6.8	5.7	5.6	6.2	0

Patient	Type of Tx	Number of infusions	Number of IEQ Tx/kg	Pre-Tx HbA1c	3 m HbA1c%	6 m HbA1c%	12 m HbA1c%	12 m insulin
26	ITA	3	9645	6.8	5.7	5.9	6.0	0
27	ITA	2	14 804	7.7	5.9	6.0	6.6	0.12
28	ITA	3	13 182	4.8	4.4	4.2	4.7	0
29	ITA	2	12 700	6.3	5.4	5.8	5.9	0
30	ITA	2	13 281	6.0	5.6	5.7	5.2	0

Subject 28, Center 3 had an artificially low HbA1c due to being on dapsone at the time of transplantation.

*Subject 2, Center 2 withdrew from the study.

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Table 3

Insulin and C-peptide response to glucose, arginine, glucose-potentiated arginine and SUITO Score at 3, 6, 12 and 24 months posttransplant. (A) Intravenous glucose tolerance test glucose (IVGTT) Glucose disposal and insulin and C-peptide secretion kinetics after stimulation with glucose. Incremental first-phase AUC and percentage over basal (means of the values from 2–10 minus basal value, divided by the basal value and expressed as percent). Kg was calculated based on the values for 10–20 min post glucose injection. (B) Arginine alone at 5mM glucose (ARG). Basal levels, incremental AUC from 2 to 5 min data and percentage of basal was calculated for plasma insulin and C-peptide after arginine stimulation. (C) Glucose potentiation of arginine-induced secretion (GPAIS) at 15 mM glucose. Basal levels, ncremental AUC from 2 to 5 min data and percentage of basal was calculated for plasma insulin and C-peptide after arginine stimulation at hyperglycemia

N = 1		**3 months n = 27**			**6 months n = 19**			**12 months n = 27**		**Nondiabetic control subjects n = 10***		
Section A: IVGTT	Basal	Incr. AUC	% Baseline	Basal	Incr. AUC	% Baseline	Basal	Incr. AUC	% Baseline	Basal	Incr. AUC	% Baseline
Insulin	59.2 ±11.9	945.8 ±172	244 ±39*	41.2±7	955 ±154.2	337±61*	39.1 ±4.5	915.6±209	300.8 ±59*	16±4	1448 ±243	1036 ±294
C-peptide	0.4 ±0.03	2.11 ±0.31*	53 ±8.4*	1.42 ±0.4**	2.45 ±0.4*	65±11*	0.42 ±0.1	1.89 ±0.43*	50 ± 11 *	0.5 ±0.1	7.3± 1.2	160±71
Kg	1.33 + 0.1			1.38 + 0.1					1.4 + 0.2	1.4 + 0.2		
		n = 27			N = 19			N = 27			n = 10	
Section B: ARG												
Insulin	63.3 ±11.9	538.7 ±60	257 ±29**	70.8±19	693.8 ±124	288.5 ±43	52.6±6***	516.9±102	247 ±49	23±4	708 ±261	446 ±106
C-peptide	0.4 ±0.1	1 ±0.12*	58±6***	0.54 ±0.1	1.36±0.2**	60.7 ±9**	0.5 ±0.1	1.12±0.2**	56±5***	0.5 ±0.1	2.7 ±0.6	125 ±29
	n = 13	n = 8	n = 13	n = 10								
Section C: GPAIS												
Insulin	150.8 ±48	2138±291**	1385 ±333*	116±36	2722 ±587**	1794±343***	147.3 ±47	3308 ±942*	746.8 ±239	168±27	6817±1527	4552 ±623
C-peptide	0.6 ±0.1	7.0±1.2***	334 ±34*	0.7 ±0.2	10.1 ±2***	368 ±78***	0.7 ±0.2	6.0±2.6***	476 ±166**	0.5 ±0.1	21.1 ±2.7	912 ±138
	N = 27	N = 19	N =27	N = 10								
Section D: SUITO	3 months			6 months			12 months		Nondiabetic control subjects			
score	49.2 + 6.0			60.6±7.3			67.5±15.0		132.5 + 93.5			

Nondiabetic subjects were used as a control group. Data are expressed as mean ± SEM.

* $p < 0.001$ for this time point to nondiabetic controls.

** $p < 0.05$ for this time point to nondiabetic controls.

*** $p < 0.01$ for this time point to nondiabetic controls.