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Assessment of β -Cell Mass and α - and β -Cell Survival and Function by Arginine Stimulation in Human Autologous Islet Recipients

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We used intravenous arginine with measurements of insulin, C-peptide, and glucagon to examine β -cell and α -cell survival and function in a group of 10 chronic pancreatitis recipients 1–8 years after total pancreatectomy and autoislet transplantation. Insulin and C-peptide responses correlated robustly with the number of islets transplanted (correlation coefficients range 0.81–0.91; $P < 0.01$ – 0.001). Since a wide range of islets were transplanted, we normalized the insulin and C-peptide responses to the number of islets transplanted in each recipient for comparison with responses in normal subjects. No significant differences were observed in terms of magnitude and timing of hormone release in the two groups. Three recipients had a portion of the autoislets placed within their peritoneal cavities, which appeared to be functioning normally up to 7 years posttransplant. Glucagon responses to arginine were normally timed and normally suppressed by intravenous glucose infusion. These findings indicate that arginine stimulation testing may be a means of assessing the numbers of native islets available in autologous islet transplant candidates and is a means of following posttransplant α - and β -cell function and survival.

Total pancreatectomy with simultaneous intrahepatic autoislet transplantation is an efficacious approach to management of

chronic, unrelenting pain and narcotic dependence for patients with chronic pancreatitis (1–3). However, information about survival of autoislet mass after transplantation is limited. Indirect measures of islet function over time after autotransplantation, such as blood levels of glucose and HbA_{1c}, have been commonly used to evaluate success, but direct and dynamic assessments that uniquely characterize β - and α -cell secretion in response to intravenous agonists are infrequently performed.

Intravenous arginine is an ideal β - and α -cell agonist that allows simultaneous examination of acute insulin (AIRarg), C-peptide (ACRarg), and glucagon (AGRarg) responses. This amino acid has the additional and important advantage of being suitable for studying secretion of these hormones when recipients are hyperglycemic, whereas the acute insulin and C-peptide responses from β -cells to intravenous glucose, a more conventional agonist, are impaired when blood glucose levels exceed 100 mg/dL and disappear altogether when blood glucose levels exceed 115 mg/dL (4,5). In this study, we used a standardized method (glucose potentiation of arginine-induced insulin secretion [GPAIS]) of intravenous arginine injection before and during intravenous glucose infusion (6,7) to quantify β -cell secretory reserve of insulin and C-peptide. The strategy of this method is to use a glucose infusion to recruit insulin granules to the extracellular β -cell

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membrane where the AIRarg and ACRarg responses occur. These and similar measures have been reported to correlate significantly with β -cell mass in animal models and humans (6,8–16). We used GPAIS to examine insulin responses in a small group of autoislet recipients 15 years ago (14). No other similar studies of autoislets have been reported. We now report data from studies of 10 new recipients to verify our previous findings and to provide novel C-peptide and glucagon measurements that have not been previously reported.

Hormonal responses to arginine in total pancreatectomy and autoislet transplantation (TP/IAT) recipients with a history of normal to near-normal blood levels of glucose (<125 mg/dL) and HbA_{1c} (<6.5%) were studied over a range of posttransplant times to address the following questions. 1) Do numbers of transplanted autoislets reliably predict the magnitudes of acute insulin and C-peptide responses? 2) Is glucagon secretion from intrahepatic α -cells normally suppressed by high circulating glucose levels? 3) When the magnitudes of β -cell responses are normalized to the numbers of islets transplanted, are they relatively the same, greater, or less than values from control subjects? 4) How do insulin responses normalized to the number of intrahepatic islets in autoislet recipients in this study compare with responses previously reported for transplanted intrahepatic alloislets in type 1 diabetic recipients?

RESEARCH DESIGN AND METHODS

Participants

Ten patients with chronic, unrelentingly painful pancreatitis who had undergone pancreatectomy and intrahepatic islet autotransplantation at the University of Minnesota ($n = 9$) and Massachusetts General Hospital ($n = 1$) were studied. Subjects with a recent history of maintaining normal to near-normal levels of fasting blood glucose (<126 mg/dL) and HbA_{1c} (<6.5%) using no insulin (except for one subject using <5 units lantus at bedtime) were recruited for the study. Subjects were 4.0 ± 0.7 years (range 1–8) post-TP/IAT at the time of study. The recipients came from different sites in the U.S. and traveled to Seattle for metabolic studies. They were lodged in a nearby hotel, fasted from 10:00 P.M., and came to the study unit at the Pacific Northwest Diabetes Research Institute at 8:00 A.M. the next morning for review of consent information provided during earlier telephone conversations. Consent forms witnessed by medical personnel were then signed. All study protocols were approved by the Western Institutional Review Board and the University of Minnesota Institutional Review Board. The participants were placed at bed rest, and indwelling percutaneous cannulas were placed in both antecubital veins for infusions and for blood draws through three-way stopcocks attached to infusions of half-normal saline to maintain patency of the cannulas. All 10 recipients and 9 healthy control subjects were studied, and all assays were performed at the Pacific Northwest Diabetes

Research Institute. TP/IAT recipients were 9/10 female sex, 35 ± 4 years of age with BMI 24.3 ± 0.7 kg/m², fasting plasma glucose 93 ± 3 mg/dL, and HbA_{1c} $5.7 \pm 0.2\%$. Control subjects were 7/9 female sex, 41 ± 4 years of age with BMI 29.8 ± 1.4 kg/m², fasting plasma glucose 96 ± 3 mg/dL, and HbA_{1c} $5.4 \pm 0.1\%$. None of these differences between islet recipients and control subjects was significant. Renal function (serum creatinine levels) was normal in all recipients and control subjects.

Surgical Procedure

The procedure of total pancreatectomy, islet isolation, and islet autotransplantation has been described in detail elsewhere (3). In brief, total pancreatectomy, splenectomy, and partial duodenectomy had been performed with small bowel reanastomosis by duodenoduodenostomy or Roux-en-Y procedure. The resected pancreas was distended by intraductal infusion of a collagenase solution and mechanically digested to release pancreatic islet tissue. Islets were infused into the portal venous circulation within 4–6 h of isolation with monitoring of portal pressure. The average total number of islets transplanted was $383,000 \pm 63,792$ (range 194,000–829,000). When elevated portal pressure prevented safe infusion of all islets, the remaining islets were dispersed in the intraperitoneal cavity ($n = 3$). These three recipients were given totals of 829,000, 460,000, and 427,000 islets and were studied 4, 7, and 1 year posttransplant, respectively. In these three recipients, hepatic and peritoneal islets given were 710,517 and 118,483, 320,473 and 139,527, and 240,231 and 186,769, respectively.

Methods

Islet Responses to Arginine

Glucose potentiation of arginine-induced insulin and C-peptide secretion and suppression of glucagon secretion was performed by giving two separate 5-g arginine pulses intravenously, one before and another at the end of a 50-min intravenous infusion of 20% dextrose at a rate of 275 mL/h. The glucose infusion was designed to maintain glucose levels at ~ 300 mg/dL. This technique potentiates insulin and C-peptide and suppresses glucagon secretion and provides insight into functional β -cell mass, as previously described (14). Blood samples were drawn at -10 , -5 , and 0 min before and at 2, 3, 4, 5, 7, and 10 min after the first pulse of arginine. AIRarg, ACRarg, and AGRarg responses to the initial arginine pulse were calculated as the mean of the three highest arginine-stimulated insulin, C-peptide, and glucagon values observed at 2–5 min after the arginine pulse minus the mean of the respective three insulin, C-peptide, and glucagon baseline values. Then the glucose infusion was started 10 min after the first arginine pulse, and further blood samples were drawn at 15, 30, 45, 55, and 60 min during the glucose infusion. The second arginine pulse was given and blood was drawn at 2, 3, 4, 5, 7, and 10 min thereafter. Acute insulin and C-peptide responses to the second arginine pulse

(AIRargMAX and ACRargMAX) were calculated as the mean of the three highest arginine-stimulated insulin and C-peptide values reached 2–5 min after the second arginine pulse minus the mean of the new baseline insulin and C-peptide values reached by minute 55 and 60 of the glucose infusion. Suppression of glucagon (AGRargMIN) during the glucose infusion was calculated in a corresponding manner. Insulin was assayed using the Millipore Human Insulin Specific RIA; glucagon was assayed using the Millipore Glucagon RIA kit; and C-peptide was assayed using the Millipore Human C-Peptide RIA kit, according to the method previously described (17).

Statistics

Intergroup comparisons were made using Student *t* test, Mann-Whitney *U*, or ANOVA, where appropriate. Correlation coefficients were calculated by Pearson product moment. All data are expressed as mean \pm SE with $P < 0.05$ considered significant.

RESULTS

Insulin responses to arginine for TP/IAT were generally lower compared with control subjects (Fig. 1A). Mean AIRarg for TP/IAT ($27 \pm 7 \mu\text{U/mL}$, $n = 10$) was not significantly less than control subjects ($53 \pm 13 \mu\text{U/mL}$, $n = 9$, $P = 0.877$), but mean AIRargMAX was less (120 ± 37 vs. 295 ± 58 , $P < 0.05$). The C-peptide responses to arginine were also generally lower compared with control subjects (Fig. 1B). Mean ACRarg for TP/IAT ($1.02 \pm 0.27 \text{ ng/mL}$) was not significantly less than control subjects (2.87 ± 0.67 , $P = 0.974$), but the mean ACRargMAX value was less (6.10 ± 1.93 vs. 11.13 ± 1.27 , $P < 0.02$). The glucagon responses to arginine (Fig. 1C) for TP/IAT and control subjects were not significantly different and were suppressed comparably during the glucose infusion (AGRarg: TP/IAT = $52 \pm 13 \text{ pg/mL}$, control = $62 \pm 6 \text{ pg/mL}$, $P = 0.463$; AGRargMIN: TP/IAT = 45 ± 8 , control = 46 ± 4 , $P = 0.906$).

Autoislet recipients received variable numbers of islets that were fewer than those conventionally assumed to be present in the normal native human pancreas (~ 1 million). Therefore, in this study, the total numbers of islets transplanted were $\sim 38 \pm 6\%$ of that assumed to be present in nondiabetic control subjects. An analysis of correlations among transplanted islet number and insulin and C-peptide secretory responses is shown in Table 1. Because fasting plasma insulin levels correlated highly with BMI ($r = 0.95$, $P < 0.001$), insulin responses are presented both as absolute values and also as percent baseline insulin values (the latter to adjust for level of endogenous insulin resistance). This percent baseline calculation was not used for C-peptide responses because basal C-peptide correlated only weakly with BMI ($r = 0.60$, $P < 0.05$). High degrees of correlation ($r = 0.81$ – 0.91) were found between the number of autoislets transplanted and AIRarg, percent basal, AIRargMAX, ACRarg, and ACRargMAX (Table 1 and Figs. 2–4). Our previously published data

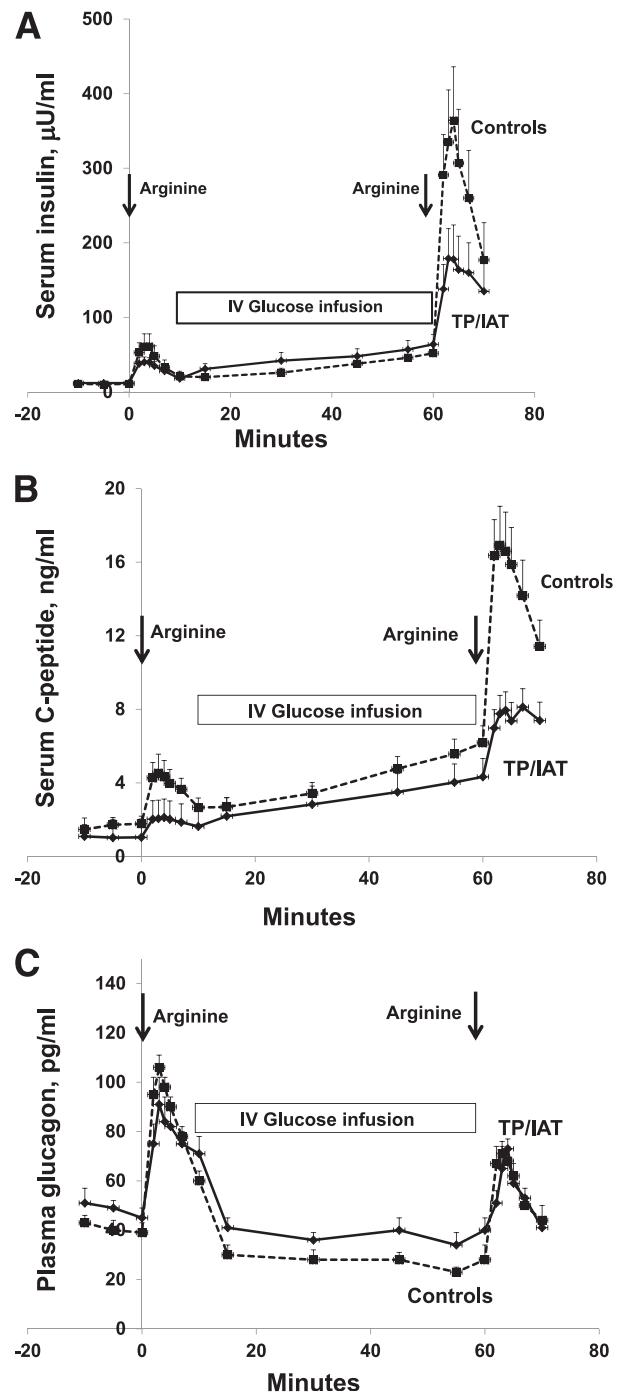


Figure 1—A: Comparison of insulin responses to intravenous arginine before (pulse 1; AIRarg) and during (pulse 2; AIRargMAX) intravenous infusion of glucose given to potentiate insulin secretion in 10 TP/IAT recipients and 9 control subjects. Both groups had responses to the first and second arginine pulses but the magnitude of AIRargMAX was significantly greater in the control group (see RESULTS for statistics). B: Comparison of C-peptide responses to intravenous arginine before (ACRarg) and during (ACRargMAX) intravenous infusion of glucose given to potentiate C-peptide secretion in 10 TP/IAT recipients and 9 control subjects. Both groups had responses to the first and second arginine pulses but only the magnitude of response was significantly greater in the control group (see RESULTS for statistics). C: Comparison of glucagon responses to intravenous arginine before and during intravenous infusion of glucose given to suppress glucagon secretion in 7 TP/IAT recipients and 9 control subjects. No differences in AGRarg were observed

Table 1—Correlation coefficients and levels of statistical significance between parameters designated x and y

x	y	r	P <
BMI	B-insulin	0.92	0.001
BMI	B-C-pep	0.60	NS
Islet #	AIRarg	0.65	0.05
Islet #	AIRarg, % Bins	0.85	0.001
Islet #	AIRargMAX	0.91	0.001
Islet #	ACRarg	0.81	0.01
Islet #	ACRargMAX	0.81	0.01
AIRargMAX	ACRargMAX	0.89	0.001

B-C-pep, basal C-peptide; % Bins, % basal insulin; B-insulin, basal insulin.

from eight subjects published 15 years ago (14), none of whom were included in the current study, are also shown in the right panels of Figs. 2 and 3 to examine the reliability of the GPAIS method to quantify β -cell responses to arginine as a reflection of transplanted islet mass.

To compare the robustness of each TP/IAT individual's insulin and C-peptide responses to the responses from control subjects, we normalized data based on the statistically significant correlations (Fig. 2A, Fig. 3A, and Fig. 4) between number of islets transplanted and magnitude of hormonal responses. Insulin response data from the current study were divided by the number of islets transplanted (response/no. islets/ 10^6) to assess the comparability of β -cell responses for TP/IAT versus control subjects. The derived values for islet number-corrected AIRarg, AIRargMAX, ACRarg, and ACRargMAX were not significantly less than the values obtained for control subjects (Figs. 5 and 6). These comparisons suggest that the individual islets transplanted into the TP/IAT recipients were secreting normal magnitudes of insulin and C-peptide in response to intravenous arginine.

DISCUSSION

This study demonstrates that the magnitudes of both insulin and C-peptide responses correlate very highly with the number of autoislets transplanted in euglycemic TP/IAT recipients. The highly significant C-peptide response correlations with β -cell mass have not been reported before. The insulin response data closely replicate information we have previously reported 15 years ago in a separate group of recipients (14), attesting to the reliability of arginine stimulation as a predictor of β -cell mass. Based on these correlations, we corrected insulin and C-peptide responses for the number of autoislets transplanted. These corrected responses in terms of timing and magnitude were not different than those observed from native pancreatic

islets in normal control subjects. These findings suggest that insulin and C-peptide responses to arginine under basal conditions or with glucose potentiation are reasonable surrogate measures for β -cell mass. However, it seems likely that not all of the transplanted islets survived, which raises the possibility that those islets that did survive could have released relatively greater amounts of hormone when stimulated with arginine than might have been anticipated. This has important implications for assessing surviving islet mass in clinical trials of islet transplantation.

Importantly, we selected autoislet recipients in whom transplantation was successful, as assessed by long-term normoglycemia without or with minimal insulin treatment. The degree of success depends greatly on the number of islets transplanted. Using criteria of C-peptide positivity and $HbA_{1c} < 7\%$, Sutherland et al. (3) reported success rates of 70–83, 85–96, and 93–98% when $< 2,500$, 2,500–5,000, and $> 5,000$ IE/kg were transplanted. It has been proposed that up to half of transplanted islets may be lost in some recipients, succumbing to factors such as hypoxia, the instant blood-mediated inflammatory response, and β -cell apoptosis (18–22). By measuring insulin secretory responses in TP/AIT recipients with moderate to high islet mass and successful outcome, we were able to establish that arginine stimulation tests allow estimation of islet mass. In addition, this normalcy of the β -cell responses when corrected for transplanted islet mass is consistent with a previous report of alloislet recipients who had normal β -cell sensitivity to glucose in the presence of impaired β -cell secretory capacity (23), together evidencing normal β -cell function of intrahepatic islets. Thus, failure to find the expected insulin responses to arginine in a TP/IAT recipient should raise concern for poor islet engraftment or later islet loss. The implications of this are critical to advancing islet transplant therapies. The arginine stimulation test may be a valuable tool in clinical trials of new approaches or adjuvant therapies in the field of islet transplantation as well as other fields of diabetes research.

The correlation between the insulin and C-peptide responses themselves was highly significant. At a practical level, this means that C-peptide measurement is a valid surrogate for insulin measurement in autoislet recipients. This is valuable information for assessing β -cell function in islet recipients taking exogenous insulin, which confounds plasma insulin measurements. This knowledge may be useful in quantifying functional status of alloislet transplants as well as whole-organ pancreas transplantation. Moreover, determination of ACRarg (or AIRarg, % B-insulin) requires < 15 min of study time, much less than oral glucose tolerance tests, and without the risk of producing hypoglycemia.

α -Cells demonstrated the expected acute responses to intravenous arginine as well as physiologic suppression during glucose infusion. This indicates that intrahepatic α -cells synthesize, store, and release glucagon normally in contrast to our previous reports of impaired ability of

between TP/IAT recipients and control subjects, and both groups had similar suppression of glucagon secretion during the glucose infusion.

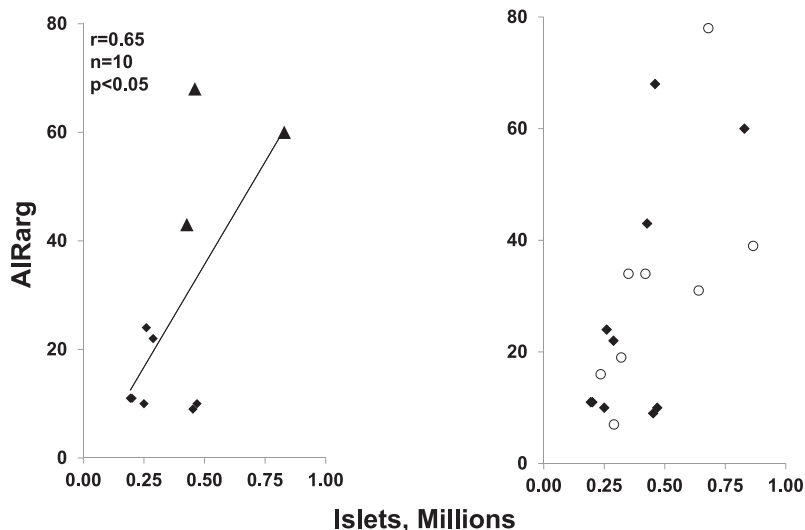


Figure 2—Correlation between number of autoislets transplanted and AIRarg. Left panel: Data from the 10 recipients currently studied demonstrating a statistically significant linear correlation. The three recipients of intraperitoneal islets are designated by the large triangles. Right panel: Superimposition of data (open circles) using the method of GPAIS previously published (14) demonstrating consistency in the AIRarg/transplanted islet number relationship using different groups of autoislet recipients.

intrahepatic α -cells to secrete glucagon during hypoglycemia (24,25). Normal glucagon responses to arginine and physiologic suppression of glucagon secretion during intravenous glucose infusion support our view that impaired intrahepatic α -cell responses to hypoglycemia are more apt to be due to microenvironmental factors within the liver (26), such as interference with hypoglycemic signaling from the general arterial circulation by intrahepatic

glucose flux from glycogenolysis, rather than intrahepatic α -cell damage or overall failure.

Insulin responses to arginine from type 1 diabetic recipients of intrahepatic alloislets also have been reported to correlate with the number of intrahepatic islets transplanted (15,16). However, the magnitudes of autoislet insulin responses corrected for numbers of islets transplanted were clearly greater than responses reported from alloislets

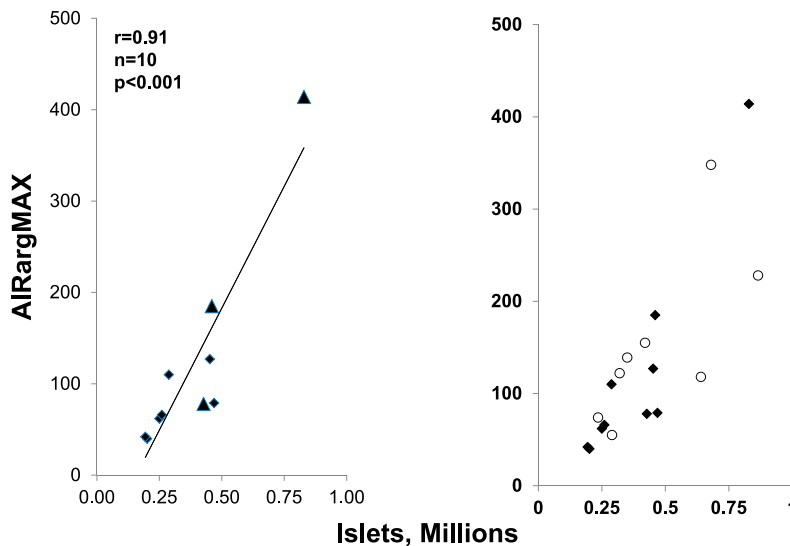


Figure 3—Correlation between number of autoislets transplanted and AIRargMAX. Left panel: Data from the 10 recipients currently studied demonstrating a much higher degree of statistical significance compared with the AIRarg data in Fig. 2. The three recipients of intraperitoneal islets are designated by the large triangles. Right panel: Superimposition of previously published AIRargMAX data (open circles) (14) demonstrating consistency of the relationship between AIRargMAX and autoislet number in different groups of autoislet recipients studied at different times.

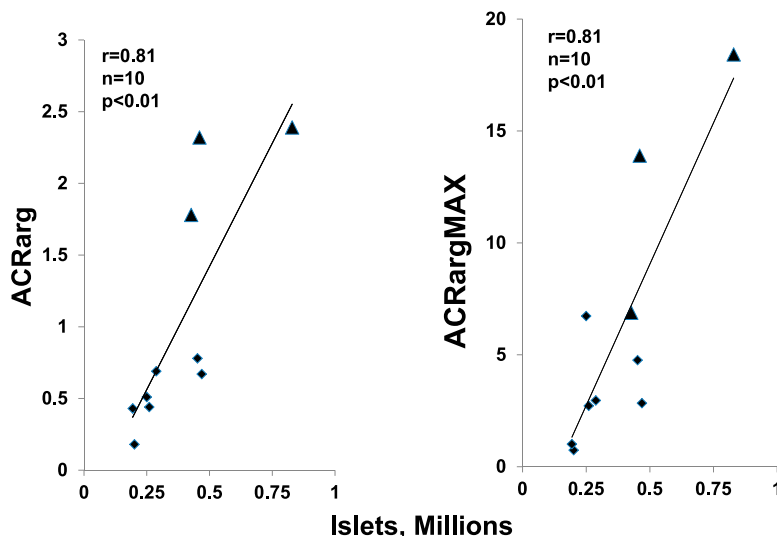


Figure 4—Correlation between number of autoislets transplanted and ACRarg and ACRargMAX. The correlations for both secretory measures with transplanted autoislet number were highly statistically significant. The three recipients of intraperitoneal islets are designated by the large triangles.

transplanted in type 1 diabetic recipients (Fig. 7). The slopes of the autoislet AIRarg and AIRargMAX correlation lines were steeper and arithmetically greater than the corresponding slopes of the alloislets. AIRarg slopes, calculated as $m = y_2 - y_1 / x_2 - x_1$, were 80 for autoislets and 16 for both published alloislet references (15,16). AIRargMAX slopes were 560 for the autoislets and 160 for the one alloislet reference reporting this value (16). Inspection of these lines reveals that for a given number of intrahepatic islets transplanted, autoislets have approximately fivefold greater AIRarg responses and 3.5-fold greater AIRargMAX responses.

This difference is not unexpected for several reasons. Alloislets run the risk of being compromised by the use of oral immunosuppressive drugs that concentrate in the liver and are known to be β -cell toxic (reviewed in Robertson [27]) and face the risk of allo- and autoimmune damage. Moreover, preparation of alloislets is more demanding than is the case for autoislets because autoislets are not always subjected to purification by Cobe cold centrifugation and they are transplanted within several hours after harvest from a living (self) donor, whereas the procurement time for alloislets prior to transplantation is generally greater and includes the risk factors of procuring islets from brain-dead donors being treated with various drugs and sustained by life support measures. Viewing these data optimistically, since the field of alloislet transplantation is steadily progressing with improved metabolic outcomes (28,29), hopefully over time alloislets will approximate the performance of autoislets and achieve a high rate of success in treatment of type 1 diabetes.

Interestingly, the high degree of correlations between β -cell function and mass in this group of autoislet recipients suggests that the degree of islet survival and level of islet function was relatively constant despite the wide

range of elapsed time since transplantation. Otherwise, the secretory values of insulin and C-peptide in the longer-term recipients would have fallen below the regression lines. Additionally, the magnitudes of insulin and C-peptide responses from the three subjects who had 14–44% of their islets placed in their peritoneal cavities (Figs. 2–4, large triangles) were indistinguishable from those of the seven recipients of intrahepatic islets only. This result suggests that human islets placed in the peritoneal cavity can survive and function. Otherwise, if intraperitoneal islets were considered nonfunctional and not included in the calculation of islet number transplanted, thus assigning a lower islet number value, the secretory response values for insulin and C-peptide for the peritoneal recipients would have fallen to the left of the regression lines and given them higher secretory values.

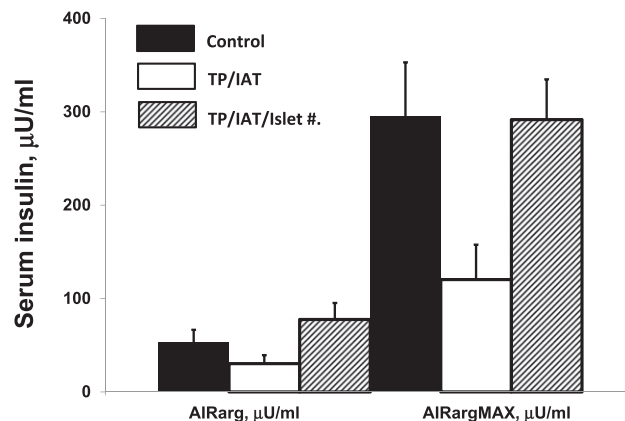


Figure 5—Comparison of AIRarg and AIRargMAX data in the control and TP/IAT groups adjusted or not adjusted for number of autoislets transplanted. No significant difference was observed between the control and TP/IAT groups when the latter data were normalized to number of islets transplanted.

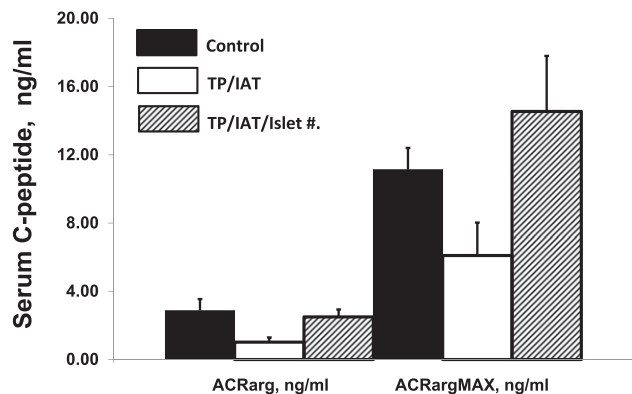


Figure 6—Comparison of ACRarg and ACRargMAX data in the control and TP/IAT groups adjusted or not adjusted for number of autoislets transplanted. No significant difference was observed between the control and TP/IAT groups when the latter data were normalized to number of islets transplanted.

Our study further suggests there would be value in performing GPAIS for patients considering TP/IAT to provide them with an estimation of their intrapancreatic β -cell mass before electing to undergo the procedure. Because there is significant variation in islet isolation success and in islet engraftment, further study is warranted to determine the utility of GPAIS to predict islet isolation outcomes or later diabetes risk. It should also provide laboratories valuable information about the efficacy of their methods of isolation and provide surgeons novel insights about their methods of pancreas procurement and transplantation techniques. Furthermore, GPAIS may present advantages over other previously studied techniques such as mixed-meal tests and intravenous

glucose tolerance testing, which are correlated with islet isolation outcomes but lack precision to predict the isolation outcome for any individual (30).

Developing imaging technologies for use in determining β -cell mass in humans has become an intense area of interest because such a measurement would be valuable for assessment for diabetes risk as well as therapeutic responses to drugs designed to improve glucose control in people with type 2 diabetes. Data from physiologic measurements, such as GPAIS, that involve β -cell agonists and insulin and/or C-peptide secretion have already been established to reproducibly provide estimates of β -cell mass (6,8–15). An advantage of physiologic studies is that they are readily available, simple, inexpensive, and include information about timing and magnitude of β -cell secretion. Since the correlation between known islet mass in autoislet recipients with functional data from studies such as GPAIS is so robust, it would seem valuable to include functional studies in research developing imaging technologies of the islet to examine how well the information from the two approaches correlate.

In summary, insulin and C-peptide responses to arginine are reasonable surrogate measures of islet mass in islet autotransplant recipients and suggest intraperitoneal islets function well. Arginine stimulation tests may be valuable for assessing patients with chronic pancreatitis prior to TP/IAT and for clinical trial end points of adjuvant therapies in islet transplantation in general. Compared with autoislet recipients, alloislet recipients have a lower magnitude of insulin secretory response/islet. This difference optimistically supports current efforts to refine technologies designed to reduce islet loss and injury during procurement of alloislets, which

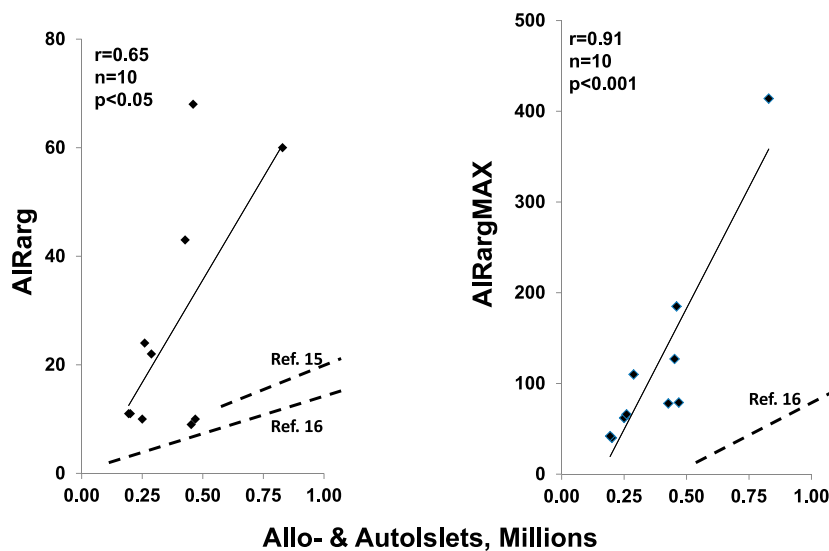


Figure 7—Comparison of the regression lines for AIrArg and AIrArgMAX for autoislets and previously published data for alloislets transplanted in type 1 diabetic recipients (15,16). The regression lines for the alloislets were less steep and had smaller slopes than the autoislets (see DISCUSSION for calculations).

may improve outcomes in allotransplantation for type 1 diabetes.

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Author Contributions. R.P.R. wrote the manuscript and performed the experiments. L.D.B. and M.R.R. performed the experiments. E.O. performed the laboratory assays. S.P. performed the experiments and the laboratory assays. M.E.P., C.S., T.D., T.P., A.N.B., D.E.R.S., G.B., and M.D.B. reviewed the manuscript and contributed to surgical and posttransplant procedures. R.P.R. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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