Improvement in β-Cell Secretory Capacity Following Human Islet Transplantation According to the CIT07 Protocol

Short title: Islet transplants and β-cell secretory capacity


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Abstract

The Clinical Islet Transplantation 07 (CIT07) protocol utilizes anti-thymocyte globulin and etanercept induction, islet culture, heparinization and intensive insulin therapy, with the same low-dose tacrolimus and sirolimus maintenance immunosuppression as in the Edmonton protocol. To determine whether CIT07 improves engrafted islet β-cell mass, our center measured β-cell secretory capacity from glucose-potentiated arginine tests at days 75 and 365 post-transplant, and compared the results to those previously achieved by our group with the Edmonton protocol, and to normal. All subjects were insulin-free with CIT07 subjects receiving fewer islet equivalents from a median 1 vs. 2 donors in Edmonton subjects. The acute insulin response to glucose-potentiated arginine ($\text{AIR}_{\text{pot}}$) was greater in CIT07 than Edmonton and less in both cohorts than normal, with similar findings for C-peptide. The CIT07 subjects who completed reassessment at day 365 exhibited increasing $\text{AIR}_{\text{pot}}$ by trend relative to day 75. These data indicate that engrafted islet β-cell mass is markedly improved with the CIT07 protocol, especially given more frequent use of single islet donors. While several peri-transplant differences may have each contributed to this improvement, the lack of deterioration in β-cell secretory capacity over time in CIT07 suggests that low-dose tacrolimus and sirolimus are not toxic to islets.
Islet transplantation is an emerging cell therapy for the treatment of type 1 diabetes (T1D) (1), particularly for those patients experiencing severe problems with hypoglycemia or who have already received a kidney transplant. The Edmonton protocol for islet transplantation established that glucocorticoid-free immunosuppression together with a subsequent islet infusion from a second donor pancreas could reproducibly render the recipient insulin-independent (2). A multi-center trial using this approach resulted in insulin-independence in 60% of recipients, although the majority of these patients returned to insulin therapy by 2 years post-transplant (3). Nonetheless, 80% of recipients maintained islet graft function as indicated by a reduction in insulin requirements and C-peptide production for the 2 years of follow-up (3). Using the Edmonton protocol, we previously reported that despite receiving almost a million islets, insulin-independent recipients had a β-cell secretory capacity only ~ 25% of normal (4). The lower functional islet β-cell mass for the numbers transplanted suggested early loss of transplanted islets before engraftment that might be due to nonspecific inflammatory and thrombotic mechanisms (5). This reduced engrafted islet β-cell mass is just at the margin of what is required to avoid hyperglycemia (6), and so likely explains the eventual return to insulin therapy experienced by the majority of recipients treated by the Edmonton protocol.

More recent induction immunosuppression protocols introduced from the University of Minnesota have incorporated peri-transplant anti-inflammatory and anti-thrombotic therapy with similar low-dose calcineurin inhibitor and mTOR inhibitor maintenance therapy as in the Edmonton protocol with improved rates of insulin-independence occurring more frequently with islets isolated from a single donor (7) and being sustained for a longer duration (8;9). The multi-
center Clinical Islet Transplantation 07 (CIT07) protocol utilizes components of the Minnesota approach including the polyclonal T cell depleting antibody rabbit anti-thymocyte globulin (rATG) and the tumor necrosis factor alpha (TNFα) inhibitor etanercept at induction, an islet culture period of 36-72 hours, full heparinization peritransplant, pentoxifylline and anticoagulation for 1 week (7), together with intensive insulin therapy for 2 months, and the same low-dose tacrolimus and sirolimus (formerly called rapamycin) for maintenance immunosuppression as in the Edmonton protocol (10). We sought to determine whether the peri-transplant changes in the CIT07 protocol improved engrafted islet β-cell mass by measuring β-cell secretory capacity from glucose-potentiated arginine (GPA) tests in CIT07 subjects transplanted at the University of Pennsylvania at 75 and 365 days after their final islet infusion, and comparing their results to those previously achieved at our center with the Edmonton protocol.

**RESEARCH DESIGN AND METHODS**

Subjects included in this study were historical and previously reported insulin-independent islet transplant recipients from the Penn-JDRF study of the Edmonton protocol (n = 5) who underwent islet transplantation between 2002 and 2003 (4;11), islet transplant recipients from the CIT07 protocol at the University of Pennsylvania (n = 11) who received islet transplants between 2008 and 2012, and normal control subjects (n = 11) studied in our laboratory between 2003 and 2011. Both study protocols were approved by the Institutional Review Board of the University of Pennsylvania, and all subjects gave their written informed consent to participate. All transplant recipients had long standing C-peptide negative type 1 diabetes complicated by hypoglycemia unawareness and frequent severe hypoglycemia events. Subjects underwent one
or two intraportal infusions of ABO-compatible islets in order to achieve insulin independence. While no attempt was made at HLA matching, each subject had a negative panel reactive antibody assessment and a negative cross-match against donor T and B lymphocytes prior to each islet infusion as described previously (12).

Induction therapy for the Edmonton protocol followed that reported by Shapiro et al. (2) and utilized the IL-2 receptor antagonist, daclizumab, 1 mg/kg on day 0 and repeated every 2 weeks for a total of 5 doses. Islets were isolated using a modified Ricordi method (13) with Liberase enzyme (Roche, Indianapolis, IN (14)) as previously described (15). Islets were cultured temporarily prior to transplantation, and infused in < 24 hours via percutaneous transhepatic portal vein catheterization in Interventional Radiology. The islet product contained 35 U/kg heparin to prevent portal vein thrombosis. Insulin therapy was discontinued post-transplant and resumed if the fasting glucose was > 125 mg/dl or post-prandial blood glucose was > 200 mg/dl. The glucose values prompting insulin therapy also served as criteria for receiving a second islet infusion (2). If a second islet infusion occurred more than 10 weeks after the first, the course of daclizumab was repeated.

Induction therapy for the CIT07 protocol followed that reported by Hering et al. (7) and included rATG up to 6 mg/kg in divided doses from day -2 to day 2 from transplant, etanercept 50 mg intravenously 1 hour pre-transplant and 25 mg subcutaneously on days 3, 7, and 10 following transplant. Pentoxifylline slow release was administered as 400 mg orally three times daily from day -2 to day 7 post-transplant (7). Islets were again isolated using a modification of the Ricordi method (13) but with Serva collagenase (Heidelberg, Germany (16)) replacing the Liberase enzyme. Islets were cultured for 36 – 72 hours in order to allow the first doses of rATG to be completed prior to transplantation, and were subsequently infused via percutaneous
transhepatic portal vein catheterization. The islet product contained 70 U/kg heparin to prevent portal vein thrombosis and prophylactic anticoagulation was continued with intravenous heparin (target partial thromboplastin time 50 – 60 seconds) for 48 hours, followed by enoxaprin 30 mg subcutaneously twice daily through day 7 (7). Subjects transitioned from subcutaneous insulin to intravenous insulin at least 2 hours prior to transplant, and were transitioned back to subcutaneous insulin the following morning. Intensive insulin therapy was maintained for 2 months following an initial islet infusion and for at least 1 month in cases of a second islet infusion. For second islet infusions, 20 mg of the IL-2 receptor antagonist basiliximab was given on days 0 and 4 from transplant in place of rATG. One previously described subject (17) presented with rATG hypersensitivity after receiving 3 mg/kg of drug and before islet infusion that was cancelled; 5 months later she underwent islet transplantation under basiliximab in place of rATG. Following each islet infusion, insulin-independence was determined at day 75 if, after tapering off insulin for more than 1 week, the fasting glucose was ≤ 126 mg/dl and a 90-minute mixed-meal tolerance test glucose was ≤ 180 mg/dl, among other criteria (10).

Maintenance immunosuppression was equivalent in both the Edmonton and CIT07 protocols, consisting of low-dose tacrolimus (12-hour blood trough target 3 – 6 µg/L) and sirolimus (24-hour blood trough target 10 – 15 µg/L for the first 3 months and 8 – 12 µg/L thereafter).

**Glucose-potentiated arginine (GPA) test.** All subjects were admitted to the University of Pennsylvania Clinical and Translational Research Center the afternoon before study and fasted overnight after 2000 for 12 h before testing. By 0700, one catheter was placed in an antecubital vein for infusions, and one catheter was placed in a hand vein for blood sampling, with the hand
placed in a thermoregulated box (~ 50ºC) or heating pad to promote arterialization of venous blood. After at least 20 min of acclimatization to the intravenous catheters, baseline blood samples were taken at -5 and -1 min before the injection of 5 g of 10% arginine over a 1-min period starting at \( t = 0 \). Additional blood samples were collected at \( t = 2, 3, 4, \) and 5 min after injection. Beginning at \( t = 10 \) min, a hyperglycemic clamp technique (18) using a variable rate infusion of 20% glucose was performed to achieve a plasma glucose concentration of ~ 230 mg/dl. Blood samples were taken every 5 min, centrifuged, and measured at bedside with an automated glucose analyzer (YSI 2300; Yellow Springs Instruments, Yellow Springs, OH) to adjust the infusion rate and achieve the desired plasma glucose concentration. After 45 min of the glucose infusion (at \( t = 55 \) min), a 5 g arginine pulse was injected again with identical blood sampling, ending at \( t = 60 \) min. It has been demonstrated that the first injection of arginine has no effect on the subsequent response to arginine using this protocol (19).

**Biochemical analysis.** All samples were collected on ice into tubes containing EDTA, trasylol, and leupeptin (Protease Inhibitor Cocktail; Sigma-Aldrich, St. Louis, MO), centrifuged at 4ºC, separated, and frozen at -80ºC until subsequent analysis. Plasma glucose was measured in duplicate by the glucose oxidase method using an automated glucose analyzer (YSI 2300; Yellow Springs Instruments). Plasma insulin and C-peptide were measured in duplicate by double-antibody radioimmunoassay (Millipore, Billerica, MA).

**Calculations and statistics.** The acute insulin and C-peptide responses to arginine (\( \text{AIR}_{\text{arg}} \) and \( \text{ACR}_{\text{arg}} \)) were calculated as the mean of the 2, 3, 4, and 5 min values minus the mean of the pre-stimulus values (18;19). Acute responses during the ~230 mg/dl glucose clamp enable
determination of glucose potentiation of arginine-induced insulin (AIR\textsubscript{pot}) and C-peptide (ACR\textsubscript{pot}) release. We have previously shown in islet transplant recipients that AIR\textsubscript{pot} is highly predictive of the maximal acute insulin response (AIR\textsubscript{max}) during a ~340 mg/dl glucose clamp ($r^2 = 0.98$, $P < 0.0001$ (20)), and so represents a valid measure of β-cell secretory capacity in this population.

All data are expressed as mean ± SE. Comparison of results across groups was performed by one-way ANOVA or Kruskal-Wallis ANOVA for non-parametric data, and when significant differences were found, comparisons between groups were performed with unpaired Student’s $t$ tests or the Mann-Whitney U test as appropriate using Statistica software (StatSoft, Inc., Tulsa, OK). Statistical significance was defined as $P < 0.05$ (two-tailed).

RESULTS

Subject characteristics. The Edmonton cohort had undergone islet transplantation between 2002 and 2003 and the CIT07 cohort between 2008 and 2012 at the Hospital of the University of Pennsylvania. The Edmonton, CIT07 and normal control groups were of comparable gender distribution, age, BMI and kidney function (Table 1). All transplant recipients were free from exogenous insulin use at the time of study 90 and 75 days following the last islet infusion for the Edmonton and CIT07 cohorts, respectively, with one subject in the Edmonton cohort initially studied 365 days post-transplant. The two cohorts of islet transplant recipients had a similarly long duration of type 1 diabetes, but the Edmonton cohort received more islet equivalents with 4/5 subjects requiring a second islet infusion compared to only 4/11 subjects in the CIT07 cohort ($P < 0.05$; Table 1). Islet donors utilized during Edmonton were older ($P < 0.05$) with isolations resulting in fewer islet equivalents per g donor pancreas ($P < 0.05$), although measures of islet
preparation purity, viability, and glucose-stimulated insulin release were not different between the Edmonton and CIT07 groups (Table 1). The HbA$_1c$ in the Edmonton cohort was higher than normal ($P < 0.01$; Table 1), but not statistically different from the CIT07 cohort that was comparable to normal. Fasting glucose was greater than normal in both the Edmonton and CIT07 cohorts ($P < 0.01$ for both; Table 1), but was not different between the Edmonton and CIT07 cohorts. Fasting insulin, but not C-peptide, was higher in the CIT07 cohort than in both the Edmonton cohort and normal group ($P < 0.01$ for both; Table 1). There was no difference in blood trough concentrations of tacrolimus or sirolimus between the Edmonton and CIT07 cohorts. One of the CIT07 subjects was converted from sirolimus to mycophenolate mofetil 500 mg twice daily with a tacrolimus trough target 6 – 9 µg/L due to the development of interstitial pneumonia (21) 4 weeks post-transplant that subsequently resolved.

**Glucose, insulin and C-peptide.** Fasting glucose, insulin and C-peptide levels given in Table 1 are the pre-stimulus levels before the first injection of arginine at the initial time of assessment. The pre-stimulus glucose during the 230 mg/dl glucose clamp was not different across the Edmonton, CIT07 and normal groups (230 ± 15 vs. 236 ± 5 vs. 236 ± 5 mg/dl). The pre-stimulus insulin and C-peptide levels during the 230 mg/dl glucose clamp provide measures of second-phase β-cell responsiveness (Fig. 1). Second-phase insulin was less in Edmonton (reported in ref. 11) than in CIT07, and in CIT07 was greater than normal (18 ± 3 vs. 40 ± 6 vs. 23 ± 3 µU/ml; $P < 0.01$ for both vs. CIT07). Second-phase C-peptide was also less in Edmonton than in CIT07, but in CIT07 was not different from normal (1.4 ± 0.4 vs. 3.7 ± 0.6 vs. 3.5 ± 0.4 ng/ml; $P < 0.05$ for both vs. Edmonton).
For the initial time of assessment in the Edmonton cohort, the previously reported acute insulin (11) and C-peptide (4) responses are given in Fig. 1A,B, and for the CIT07 cohort in Fig. 1C,D. The acute insulin response to arginine (AIR<sub>arg</sub>) was not different across the groups (15 ± 2 vs. 28 ± 4 vs. 28 ± 3 µU/ml) while the acute insulin response to glucose-potentiated arginine (AIR<sub>pot</sub>) was less in the Edmonton compared to the CIT07 cohort (29 ± 3 vs. 58 ± 7 µU/ml; P < 0.05), and less in both transplant cohorts than normal (143 ± 15 µU/ml; P < 0.01 for both comparisons). In Fig. 1B, the acute C-peptide response to arginine (ACR<sub>arg</sub>) was lower in Edmonton than in CIT07 or normal (0.5 ± 0.1 vs. 1.3 ± 0.2 vs. 1.5 ± 0.2 ng/ml; P < 0.01 for both comparisons), and the acute C-peptide response to glucose-potentiated arginine (ACR<sub>pot</sub>) was less in Edmonton than in CIT07 (1.1 ± 0.2 vs. 2.2 ± 0.3 ng/ml; P < 0.05), and less in both transplant cohorts compared to normal (6.4 ± 0.8 ng/ml; P < 0.01 for both comparisons).

**Reassessment at 365 days.** For all islet recipients who underwent reassessment, there was no difference in the pre-stimulus glucose during the 230 mg/dl glucose clamp (228 ± 5 vs. 224 ± 5 mg/dl) with the between subject difference < 2% of the mean. Of the Edmonton protocol treated subjects, one returned to insulin shortly after initial study and lost all islet graft function by 365 days (-□-), one returned to insulin at 9 months and received a second islet infusion (-○-), two returned to insulin at 11 months and exhibited declines in β-cell secretory capacity at 365 days (-Δ-,-◊-), and the one initially studied at 365 days remained insulin-independent at 730 days with stable β-cell secretory capacity (-◇-; Fig. 2A). One of these subjects (-◊-) had discontinued sirolimus because of associated colitis and was previously reported to have developed islet donor specific alloantibodies coincident with the decline in β-cell secretory capacity (22). All of the CIT07 protocol treated subjects have remained insulin-independent, with all nine who have
completed the day 365 assessment demonstrating stable β-cell secretory capacity (Fig. 2B). Fig. 3A & B demonstrate reproducibility of the hyperglycemic clamp conditions from day 75 to day 365 in the CIT07 cohort. In Fig. 3C, the AIR_{arg} at days 75 and 365 remained normal (29 ± 5 vs. 35 ± 6 vs. 28 ± 3 µU/ml), and the AIR_{pot} increased by trend from day 75 to 365 (60 ± 9 vs. 79 ± 11 µU/ml; \( P = 0.05 \)), although both were less than normal (143 ± 15 µU/ml; \( P < 0.01 \) for both comparisons). In Fig. 3D, similar results are seen for ACR_{arg} (1.3 ± 0.2 vs. 1.4 ± 0.3 vs. 1.5 ± 0.2 ng/ml) and ACR_{pot} (2.4 ± 0.4 vs. 2.7 ± 0.5 vs. 6.4 ± 0.8 ng/ml; \( P < 0.01 \) for both comparisons), again exhibiting a notable absence of deterioration with time.

**DISCUSSION**

The present study demonstrates a significant improvement in β-cell secretory capacity following islet transplantation using the CIT07 compared to historical use of the Edmonton protocol. Because β-cell secretory capacity provides the best estimate of functional β-cell mass (23;24), these results provide evidence for improved islet engraftment with the CIT07 protocol, an even more remarkable finding when one considers that fewer islets were transplanted, and most often from a single donor pancreas, using the CIT07 protocol. Importantly, and in contrast to past experience with the Edmonton protocol, there was a distinct absence of deterioration of the β-cell secretory capacity in the CIT07 protocol treated subjects between 75 and 365 days post-transplant. These findings suggest that a reserve capacity for insulin secretion above that necessary to achieve initial insulin independence is required to maintain long-term islet graft function. This reserve capacity may protect islets from excessive metabolic demand inducing endoplasmic reticulum stress (25) or amyloid deposition (26) that have been described in pre-clinical islet transplant models. Moreover, this sustainable β-cell secretory capacity in the CIT07
protocol subjects is evident despite the use of the same low-dose tacrolimus and sirolimus maintenance immunosuppression as in the Edmonton protocol. We have previously shown that the β-cell secretory capacity is fully normal in uncomplicated whole pancreas transplant recipients who have received a 100% of an islet β-cell mass despite treatment with tacrolimus-based immunosuppression (11). Thus, these current data are consistent with the interpretation that modern dosing of currently available immunotherapy is not toxic to islets.

Since the maintenance immunosuppression therapy was identical under both protocols, the superior β-cell secretory capacity with CIT07 is best explained by its peri-transplant differences from the Edmonton protocol. rATG has proved more efficacious at suppressing alloimmunity than an IL-2 receptor antagonist (27), and has been shown to affect autoimmunity in recent onset type 1 diabetes (28). However, its use in islet transplantation had previously been limited by the requirement for glucocorticoid prior to the first dose to ameliorate symptoms of cytokine release from lysed T lymphocytes, both known to be detrimental to islets by increasing secretory demand and by direct toxicity, respectively (29;30). With the introduction of a period of islet culture, Hering et al. (7) showed that by administering the first dose of rATG with glucocorticoid > 36 hours prior to islet infusion, a high rate of single donor insulin-independence could be achieved. In addition, initiation of the TNF-α inhibitor etanercept prior to islet infusion is also likely to mitigate any residual cytokine effects or non-specific inflammation related to intraportal islet delivery (31). In fact, Bellin et al. demonstrated that induction regimens based on a T lymphocyte depleting antibody resulted in superior long-term insulin independence when compared with use of an IL-2 receptor antagonist, but only when combined with a TNF-α inhibitor (9). It is also possible that peri-transplant administration of pentoxifylline, which can inhibit TNF-α production (32), may contribute to anti-inflammatory efficacy. Our data support
that the prolonged insulin-independence reported with combined T lymphocyte depletion and TNF-α inhibitor is at least in part attributable to improved engraftment of a sufficient islet β-cell mass to sustain long-term function.

Another peri-transplant difference in the CIT07 protocol is the extension of anticoagulation beyond the islet infusion procedure. As isolated islets express tissue factor that may induce an instant blood mediated inflammatory reaction (33;34), anticoagulation beyond what is required to prevent portal vein thrombosis may further decrease localized inflammation and microthrombus formation during islet revascularization and engraftment. Koh et al. (35) showed by multivariate analysis that the combined use of peri-transplant infusions of heparin and insulin was associated with a higher rate of single donor insulin independence. In CIT07, the peri-transplant heparin and insulin infusions were followed by low-molecular weight heparin until day +7 and intensive insulin therapy for 2 months before attempting to taper. Because complete functional recovery of transplanted islets is not expected for several weeks after transplantation (reviewed in (36)), we believe that intensive insulin therapy to avoid glucotoxicity (37;38) and reduce β-cell demand during this relatively hypoxic period prior to revascularization is essential to optimizing islet survival during the engraftment process. While this study cannot determine to what extent each of these peri-transplant differences may have contributed to the improved β-cell secretory capacity with the CIT07 protocol, the findings nonetheless demonstrate that marked improvements in islet β-cell engraftment may be achieved through refinements in peri-transplant management.

An important limitation to the current study is that the comparison of CIT07 protocol subjects to a historical group treated with the Edmonton protocol does not account for immeasurable confounding factors that were different during the two periods of transplantation
and may have affected the results. One likely confounder is potential differences in the quality rather than quantity of the islet preparations. While there was no difference in the \textit{in vitro} purity, viability, or glucose-stimulated insulin release measures between the islet preparations transplanted during the Edmonton compared to the CIT07 period, the younger donor age and modestly increased islet yield per gram of donor pancreas in the CIT07 group might indicate higher quality pancreata and/or more efficient islet isolation related to changes in the available collagenase enzymes used for digestion. Indeed, other reports have demonstrated better \textit{in vivo} islet function results with use of younger (< 40-45 years of age) relative to older donors (39;40), and higher islet yield per gram donor pancreas with the newer collagenase enzymes used in CIT07 compared to that used with the Edmonton protocol (41). Finally, the introduction of a pre-transplant islet culture period with the CIT07 protocol may have resulted in a less immunogenic islet graft as previous work has demonstrated an effect of islet culture on decreasing the number of passenger leukocytes and islet expression of class I HLA (42).

Interestingly, fasting and second-phase insulin, but not C-peptide, levels were increased in CIT07, a finding not present in the Edmonton subjects. We (43) and others (44) have shown that insulin sensitivity is normal in islet transplant recipients receiving low-dose tacrolimus and sirolimus immunosuppression, a finding confirmed in our CIT07 subjects by both direct and modeled measures (manuscript under review), and so insulin resistance leading to increased insulin secretion cannot explain this finding. Moreover, the normal fasting and second-phase C-peptide levels in the CIT07 subjects supports an absence of increased insulin secretory demand on the β-cell, and rather suggests a decrease in insulin clearance. A prior study that assessed hepatic extraction of insulin from intraportally transplanted islets did not detect a difference from normal (45), although the number of subjects studied was small, and similar to our Edmonton
protocol group, likely had a low engrafted islet β-cell mass that may not secrete a sufficient quantity of insulin to detect variations in hepatic extraction. In a canine auto-islet transplant model, treatment with sirolimus was associated with a 13% reduction in insulin clearance and no change in insulin sensitivity (46), a modest effect consistent with our results. Whether a potential decrease in hepatic insulin extraction in human islet recipients may be due to sirolimus or the intrahepatic site of transplantation requires further study.

In conclusion, β-cell secretory capacity, a measure of functional islet β-cell mass, is markedly improved with current use of the CIT07 protocol. These superior functional results over what has been previously reported were achieved with a lower number of islets, more often from a single donor pancreas, and have been stable for 1 year post-transplant. Reducing the number of donor pancreata required to achieve insulin-independence is important since the use of multiple donors with a greater number of HLA class I mismatches appears to be associated with an increased risk for HLA sensitization should a reduction or discontinuation of immunosuppression be required in the future (12;47). While several peri-transplant differences between the present CIT07 and historical Edmonton protocols may have each contributed to the improved engrafted islet β-cell mass, the lack of deterioration in β-cell secretory capacity over time in CIT07 suggests the achievement of a sufficient reserve capacity for insulin secretion necessary to resist metabolic exhaustion of the islet graft, as well as any purported toxicity from the required immunosuppression.

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M.R.R. designed and conducted the study, researched data, and wrote the manuscript, C.L. participated in the conduct of the study, researched data, and revised the manuscript critically for important intellectual content, R.D.S.-G., S.A.S., K.V., M.K., Z.M., E.M., M.P., C.D.-B., C.F., and A.J.C. participated in the conduct of the study, researched data, and reviewed/edited the manuscript, C.F.B. contributed to the design of the study and the discussion, and reviewed/edited the manuscript, E.T.L.P. contributed to the design of the study, participated in its conduct, researched data, and revised the manuscript critically for important intellectual content, and A.N. designed and conducted the study, researched data, and revised the manuscript critically for important intellectual content. M.R.R. is the guarantor of this work and, as such, takes full responsibility for the work as a whole, including the study design, access to data, and the decision to submit and publish the manuscript.
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factor by pancreatic islet cells as a trigger of detrimental thrombotic reactions in clinical islet transplantation. *Lancet* 360:2039-2045, 2002


FIG. 1. Plasma insulin \((A,C)\) and C-peptide \((B,D)\) levels in response to bolus injections of arginine \((\textit{arrows})\) administered under fasting and 230 mg/dl hyperglycemic clamp conditions at day 90 post-transplant in the Edmonton \((A,B)\) and day 75 post-transplant in the CIT07 \((C,D)\) protocol subjects. The Edmonton cohort underwent islet transplantation between 2002 and 2003, and the CIT07 cohort between 2008 and 2012. The insulin (ref. 11) and C-peptide (ref. 4) data for the Edmonton group have previously been reported. The gray shaded area gives the 95% confidence interval for levels in the normal control group.

FIG. 2. \(\beta\)-cell secretory capacity measured as the acute insulin response to glucose-potentiated arginine \((\text{AIR}_{\text{pot}})\) in the Edmonton \((A)\) and CIT07 \((B)\) protocol subjects over time post-transplant. The Edmonton cohort underwent islet transplantation between 2002 and 2003, and the CIT07 cohort between 2008 and 2012. The hashed area gives the 95% confidence interval for the \(\beta\)-cell secretory capacity in the normal control group.

FIG. 3. Plasma glucose \((A)\) and glucose infusion rates \((B)\) during conduct of the glucose-potentiated arginine test, and insulin \((C)\) and C-peptide \((D)\) levels in response to bolus injections of arginine \((\textit{arrows})\) administered under fasting and 230 mg/dl hyperglycemic clamp conditions at day 75 and day 365 post-transplant in the CIT07 protocol subjects. The gray shaded or hashed areas give the 95% confidence interval for levels in the normal control group.
TABLE 1
Characteristics of islet transplant recipients at the time of initial glucose-potentiated arginine testing following their final islet infusion, their islet preparations, and control subjects

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Edmonton Cohorta</th>
<th>CIT07 Cohort</th>
<th>Normal Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>3/2</td>
<td>4/7</td>
<td>5/6</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>44 ± 4</td>
<td>46 ± 3</td>
<td>41 ± 2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21 ± 1</td>
<td>23 ± 1</td>
<td>24 ± 1</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.9 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.9 ± 0.0</td>
</tr>
<tr>
<td>TID duration (yrs)</td>
<td>34 ± 3</td>
<td>30 ± 4</td>
<td>—</td>
</tr>
<tr>
<td>IE transplanted</td>
<td>918,971 ± 131,104*</td>
<td>694,895 ± 48,498</td>
<td>—</td>
</tr>
<tr>
<td>IE/kg</td>
<td>14,313 ± 2237*</td>
<td>9,480 ± 706</td>
<td>—</td>
</tr>
<tr>
<td>Islet donor age (yrs)b</td>
<td>51 ± 4*</td>
<td>37 ± 4</td>
<td>—</td>
</tr>
<tr>
<td>Islet donor BMI (kg/m²)b</td>
<td>34 ± 5</td>
<td>29 ± 1</td>
<td>—</td>
</tr>
<tr>
<td>IE/g pancreas b</td>
<td>4007 ± 142*</td>
<td>5312 ± 353</td>
<td>—</td>
</tr>
<tr>
<td>Islet preparation purity (%)b</td>
<td>~ 95</td>
<td>~ 93</td>
<td>—</td>
</tr>
<tr>
<td>Islet preparation viability (%)b</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Islet stimulation index,b,c</td>
<td>1.7 ± 0.4</td>
<td>1.7 ± 0.1</td>
<td>—</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6.1 ± 0.2**</td>
<td>5.6 ± 0.1</td>
<td>5.3 ± 0.2</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>100 ± 6**</td>
<td>104 ± 4**</td>
<td>81 ± 2</td>
</tr>
<tr>
<td>Fasting insulin (µU/ml)</td>
<td>7 ± 1</td>
<td>13 ± 1‡</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>Fasting C-peptide (ng/ml)</td>
<td>0.78 ± 0.18</td>
<td>1.23 ± 0.14</td>
<td>1.13 ± 0.17</td>
</tr>
<tr>
<td>Tacrolimus (µg/L)</td>
<td>3.8 ± 0.3</td>
<td>5.3 ± 0.7</td>
<td>—</td>
</tr>
<tr>
<td>Sirolimus (µg/L)</td>
<td>12.5 ± 1.2</td>
<td>10.6 ± 1.0</td>
<td>—</td>
</tr>
</tbody>
</table>

Data are means ± SE. *P < 0.05 for comparison to the CIT07 cohort; **P < 0.01 for comparison to the control group; ‡P < 0.01 for comparison against both the Edmonton cohort and control group. IE, islet equivalents whereby an IE approximates a standard islet diameter of 150 µm; IE/kg, IE transplanted per kg recipient body weight; IE/g, IE isolated per g donor pancreas weight.

aDemographic, insulin (ref. 11) and C-peptide (ref. 4) data were previously reported for this historical cohort of Edmonton treated subjects.
bIn cases where 2 islet infusions occurred a weighted averaged of the islet characteristic was used.
cIslet stimulation index is based on a standardized in vitro static incubation glucose-simulated insulin release assay (described in refs. 39 – 41).
To convert to mmol/mol, multiply by 10.93 and subtract 23.50.
For Peer Review Only

**CIT07 Protocol (n = 11), transplanted 2008-2012**

**Edmonton Protocol (n = 5), transplanted 2002-2003**

**Normal Range (n = 11)**

**MINUTES**

230 mg/dl hyperglycemic clamp

**ARGinine (5 g)**

**INSULIN (μU/ml)**

**C-PEPTIDE (ng/ml)**