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Correction of insulin sensitivity and glucose disposal after pancreatic islet transplantation: preliminary results

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Abstract

Aims: Pancreatic islet transplantation (PIT) represents a potential curative treatment for patients with type 1 diabetes, but only 10–15% of patients remain insulin independent 5 years post-transplant. It is not known whether intrinsic insulin resistance exacerbated by immunosuppression plays a pivotal role in low graft survival. The study objective was to understand the changes in insulin resistance, glucose effectiveness (S_g) and free fatty acid dynamics (FFAd) before and after PIT.

Methods: Insulin sensitivity index (S_i), S_g and FFAd were measured before and after PIT in 10 lean patients, 8 of whom reached insulin independence. Modified Bergman minimal model of frequently sampled intravenous glucose tolerance tests were performed pretransplant and at 12 months post-transplant. Nine non-diabetic control (NDC) subjects matched by age, gender and BMI were used.

Results: Pretransplant S_i and S_g were $3.5 \pm 0.8 \times 10^{-5}/\text{min}/(\text{pmol/l})$ and $0.74 \pm 0.24 \times 10^{-2}/\text{min}$, respectively. S_i was significantly lower than matched NDCs [$10.8 \pm 0.6 \times 10^{-5}/\text{min}/(\text{pmol/l})$, $p < 0.004$]; S_g did not reach statistical significance ($1.27 \pm 0.22 \times 10^{-2}/\text{min}$, $p = 0.25$). Compared to pretransplant values, mean post-transplant S_i and S_g were $9.6 \pm 1.3 \times 10^{-5}/\text{min}/(\text{pmol/l})$ and $1.28 \pm 0.22 \times 10^{-2}/\text{min}$, respectively, indicating significant improvement for S_i but not S_g ($p = 0.008$ and $p = 0.06$). Twelve-month post-PIT compared to NDC values were not significantly different ($p =$

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Conflicts of Interest

David Hirsch conducted and collected data, analysed the literature and drafted the manuscript. Jon Odorico was responsible for the design of the study, analysed the literature and drafted the manuscript. Nancy Radke was responsible for the design of the study, conducted and collected data. Matthew Hanson conducted and collected data, analysed the literature and drafted the manuscript. Juan Sebastian Danobeitia conducted and collected data, analysed the literature and drafted the manuscript. Debra Hullett conducted and collected data, analysed the literature and drafted the manuscript. Rodolfo Alejandro conducted and collected data and analysed the literature. Camillo Ricordi conducted and collected data and analysed the literature. Luis Fernandez was responsible for the design of the study, conducted and collected data, analysed the literature and drafted the manuscript.

The authors have nothing to disclose.

0.58 and 0.97, respectively). In addition, fractional disposal rate for FFA which directly depends on the endogenous insulin release (10–20 min) nearly normalized after PIT ($p = 0.06$).

Conclusion: These preliminary findings demonstrate that PIT can restore glucose disposal and insulin sensitivity and partially correct glucose effectiveness and FFAd.

Keywords

glucose metabolism; insulin resistance; insulin secretion; PIT

Introduction

Pancreatic islet transplantation (PIT) is a minimally invasive option to restore normal glycaemic control in patients with type 1 diabetes (T1D). The Edmonton protocol achieved unprecedented short-term insulin independence (II) at several centres [1]. Challenges that became apparent included the requirement for multiple donors to achieve II and its short duration [2,3]. Most patients retain some graft function 5 years after PIT, measured by fasting C-peptide. Factors contributing to loss of II may include: (i) instant blood-mediated inflammatory response contributing to inadequate PIT engraftment; (ii) post-operative glucotoxicity; (iii) exposure of intrahepatic islets to high immunosuppression levels; (iv) islet auto- or allo-immunity response, ultimately inducing ‘islet exhaustion’ and decreased functional β -cell reserve over time.

Although typically associated with type 2 diabetes [4,5], insulin resistance has been documented in T1D [6–8]. Insulin resistance in pre-T1D predisposes to rapid disease progression in individuals with positive islet auto-antibodies [9] and has been associated with a lower frequency of the ‘honeymoon phase’ in newly diagnosed T1D patients. Insulin resistance is also an independent risk factor for development of macro- and microvascular complications [10–12] and may contribute to β -cell stress in PIT recipients.

Lipolysis in adipose tissue is the process by which stored triglycerides are hydrolysed to yield glycerol and fatty acid. Insulin, vasopressin and nicotinic acid, among others, are widely recognized as inhibitors of lipolysis [13]. In conditions associated with insulin resistance such as overt type 2 diabetes mellitus, non-alcoholic fatty liver disease and lipoprotein lipase defects, plasma free fatty acid (FFA) concentration can be markedly elevated. FFA also inhibits glucose utilization. Improved insulin resistance and increased insulin secretion may parallel reduction of FFA.

To better understand whether changes in insulin resistance are associated with the short-term success of PIT, estimation of insulin sensitivity (S_I), glucose effectiveness (S_g) and determination of FFA concentration after intravenous (I.V.) glucose administration were calculated before and after PIT. Area under the curve (AUC) for insulin and C-peptide secretion were also calculated during the first 10 min of the frequently sampled intravenous glucose tolerance test (FSIVGTT) to understand the changes in insulin and C-peptide release upon glucose stimulation.

Materials and Methods

Subjects

Potential PIT recipients (age 18–65 years, T1D >5 years) were recruited. General inclusion criteria consisted of T1D already requiring immunosuppression for a transplant (often kidney) or with labile diabetes including life-threatening hypoglycaemia and/or ketoacidosis.

Ten T1D subjects with long-standing C-peptide-negative disease were placed on the waiting list for PIT (Table 1). All patients (islet transplant alone and islet after kidney transplant) were treated with the same immunosuppressive therapy based on slight modification of the already published immunosuppressive protocol by Edmonton [14]. In brief, patients were given an IL-2 receptor blockade at the time of transplantation at a dose of 1 mg/kg every 14 days for five consecutive doses. Steroid-free immunosuppressive therapy consisted of a maintenance combination of tacrolimus and sirolimus at levels of 2–5 ng/ml and 10–14 ng/ml, respectively, for the first year. Four patients with well-functioning kidney allografts were free of corticosteroids for a minimum of 9 months at the time of enrollment in the study. Recipients of islet transplantation alone (ITA) or islet after kidney transplantation (IAK) were managed with similar levels of sirolimus and calcineurin inhibitors in the post-islet transplant period. Patients who were on maintenance therapy with mycophenolate mofetil were maintained without dose modifications after enrollment. Two islet transplant recipients in the islet transplant alone subgroup were placed on mycophenolate mofetil or mycophenolic acid because they were considered at greater immunological risk for rejection. Nine non-diabetic control (NDC) subjects matched for BMI, gender and age underwent metabolic testing. These included six historical controls [15] and three contemporaneous controls who underwent metabolic testing at the University of Wisconsin (UW). This study was approved by UW and University of Miami Health Sciences Institutional Review Boards (HS IRB), with informed consent obtained from all subjects.

All tests were performed at UW Hospital General Clinical Research Center and Transplant Clinic and at the Metabolic Laboratory of the Diabetes Research Institute, University of Miami as per HS IRB-approved protocols. Subjects fasted overnight (12 h) before testing. Insulin-dependent subjects withheld long-acting insulin for 24 h and short-acting insulin for 12 h before testing. If required, I.V. insulin was administered overnight to maintain the blood glucose concentration between 100 and 150 mg/dl (5.5–8.3 mmol/l) and was discontinued 45 min before testing. All other medications were withheld on the morning of study. The morning of testing, one additional catheter was placed retrograde in a contralateral hand vein for blood sampling and the hand placed in a thermoregulated box (50 °C) to promote optimal arterialization of venous blood.

The study was originally designed to acquire preand post-transplant metabolic data for calculations and longitudinal comparison. Only 6 of 10 post-PIT subjects (ITA n = 4 and IAK n = 2) were available for metabolic testing pretransplant, because four patients were called for transplant before their baseline metabolic testing was performed. Eight were available 12 months post-transplant. Two patients were lost to follow-up 6 months post-transplant. At each testing time point, blood samples were collected for analysis of glucose, insulin and C-peptide. Whole blood glucose was measured immediately using a YSI 2300

Stat Glucose Analyzer (Yellow Springs Instruments, Yellow Springs, OH, USA). Serum was collected and analysed to determine insulin and C-peptide concentrations by enzyme-linked immunosorbent assay (ELISA) (Millipore, Bellerica, MA, USA) or RIA (Linco Research, St Charles, MO, USA).

Metabolic Testing

FSIVGTT for Determination of S_i and S_g

S_i and S_g were reported based on the single compartment model previously described by Bergman [4]. Briefly, following baseline blood sampling at -15, -10 and -5 min, 300 mg/kg of dextrose (50% dextrose in water) was infused at time 0 over 2 min. Samples were collected at 2, 3, 4, 5, 6, 8, 10, 14, 16 and 19 min. At time 20 min, 0.24 units/kg of regular human insulin was infused over 5 min [16,17]. Further samples were collected at times 22, 23, 24, 26, 30, 33, 36, 40, 50, 60, 70, 80, 100, 120, 140, 160 and 180 min. Each sample was analysed for glucose and insulin content, with data analysed using the MLAB computer program (Civilized Software, Inc., Silver Springs, MD, USA) and an MINMOD script.

Incremental AUC for acute-phase insulin (AIR_{GLU}) and C-peptide (ACR_{GLU}) response to I.V. glucose, a measure of β -cell function, was calculated from the first 10 min of the FSIVGTT. AUC was calculated by the trapezoidal rule with the mean of the baseline values subtracted. Glucose disappearance rate, $K_g = \ln [\text{glucose}]/\text{min} \times 100$, was calculated as the slope of the natural log of glucose values between 10 and 20 min with least-squares linear regression using computer software. To maintain the same units, in this calculation the value of AIR_g was divided by 10.

Disposition index (DI) was calculated as the product of AIR_g and S_i which represents insulin-mediated glucose uptake, because of the hyperbolic relationship between S_i and β -cell function. The basal insulin component of S_g is basal insulin effect (BIE) and is calculated as the product of basal insulin (I_b) and the S_i index, representing the basal insulin component of S_g . Glucose effectiveness at zero insulin (GEZI), a more precise description of Π glucose disposal than S_g , was calculated based on the difference between S_g and BIE: $GEZI = S_g - (S_i \times I_b)$.

Euglycaemic-hyperinsulinaemic clamp studies on the initial six control subjects were carried out using the euglycaemic-hyperinsulinaemic technique as previously described [18,19]. The calculated M value was converted to S_i values as previously published [17,20] to establish appropriate comparison with transplanted patients and contemporaneous controls.

FFA analysis (Wako Chemical USA, Inc., Richmond, VA, USA) was performed in six PIT recipients and in a subgroup of three NDC subjects. Incremental AUC for FFA (AUC_{FFA}) was calculated between 0 and 180 min after glucose administration and analysed according to the model of Sumner et al. [21], in which three phases of FFA dynamics were described. The first phase consists of an extension of baseline FFA levels for approximately 10 min and is calculated as time from $t = 0$ to initial sustained decrease in FFA. The second phase is characterized by a suppression of FFA levels until a nadir and is evaluated by the fractional disposal rate (FDR) of FFA, calculated as the slope of log-transformed FFA values between

10 and 40 min using least-squares linear regression. AUC for FFA between 10 and 80 min was also calculated and compared among the pretransplant, post-transplant and control groups. The third phase is the period from the nadir to the time FFA levels return to baseline levels. In NDC, the dynamics of these three phases were the same during both standard and insulin-modified FSIVGTT, suggesting that a normal endogenous insulin response to the injection of glucose at $t = 0$ min achieves maximal FFA suppression without added effect from the exogenous insulin administered at $t = 20$ min [22].

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 with post-testing, except where only two groups were compared by two-tailed Student's t -test. Pearson's correlation coefficient and partial correlation coefficients were estimated. The influence on the K_g index of the variables studied was estimated using stepwise multiple regression analysis. Results are shown as mean $\pm$ s.e.m. Significance was established at a value <0.05 .

Results

Demographics

No differences were seen when demographics were compared between PIT recipients and NDC with the exception of HbA1c ($p < 0.001$) and insulin requirement (Table 1).

PIT Subject Characteristics

Demographics of the first 10 PIT subjects at UW and their respective controls are summarized in Table 1 and transplant details are summarized in Table 2. Eight patients achieved II. Mean follow-up since the first islet infusion was 58.9 ± 22 months (range 28–89). Average islet equivalents infused were $22\,746 \pm 6289$ IEQ/kg body weight. Six of 10 patients remained II after the first year post-transplant. Six patients were followed with metabolic testing for 24 months after last infusion. Two subjects never achieved II despite initially reduced insulin requirement and decrease in frequency of hypoglycaemic events and HbA1c values. Of the eight subjects achieving II, one is awaiting a third islet infusion. Of the six followed for 1 year after last infusion, three remained II for more than 3 years (figure 1). No hypoglycaemic unawareness episodes after transplant have been observed and patients remain C-peptide positive with similar weights to the pretransplant period.

HbA1c

Improvement in HbA1c was observed after PIT, from a pretransplant mean of $7.2 \pm 0.3\%$ to $5.6 \pm 0.5\%$ ($p = 0.02$) 3 months post-transplant. Eight of 10 patients reached normal HbA1c values at 3 months post-PIT. Although a statistical improvement persisted throughout the follow-up period, a non-significant trend towards slowly rising HbA1c was observed.

FSIVGTT

During FSIVGTT, serum glucose rose comparably in PIT recipients before and after PIT compared to NDC (figure 1). However, their AUC for glucose disappearance and glucose disappearance rate (K_g) was quite different. AUC for glucose disappearance was significantly lower in the pretransplant group compared to post-transplant and NDC groups ($p = 0.004$ and 0.009 , respectively). No differences were seen 12 months after PIT when AUC for glucose disappearance was compared to control ($p = 0.73$). The glucose disappearance rate was also statistically significantly different between the pre- and post-transplant groups and their respective controls. For PIT recipients, the pretransplant K_g value was 0.96 ± 0.03 , compared to 1.25 ± 0.09 at 12 months post-PIT and 1.59 ± 0.15 for NDC ($p = 0.05$ and 0.004 , respectively, in relation to pretransplant value). No statistically significant difference was observed between subjects 12 months post-PIT compared to NDC ($p = 0.08$).

Prior to PIT, diabetic subjects exhibited lower S_i and S_g than normal control subjects (figure 1). Pretransplant S_i in six diabetic subjects was $3.5 \pm 0.8 \times 10^{-5}/\text{min}/(\text{pmol/l})$; in controls, $10.8 \pm 0.6 \times 10^{-5}/\text{min}/(\text{pmol/l})$ ($p < 0.0004$). S_g in pretransplant subjects with diabetes was lower ($0.74 \pm 0.24 \times 10^{-2}/\text{min}$) compared to control subjects ($1.27 \pm 0.22 \times 10^{-2}/\text{min}$; $p = 0.21$). Post-transplant, S_i and S_g increased to $9.6 \pm 1.3 \times 10^{-5}/\text{min}/(\text{pmol/l})$ and $1.28 \pm 0.22 \times 10^{-2}/\text{min}$, respectively, compared to pretransplant values ($p = 0.003$ and 0.12 , respectively). When post-transplant S_i and S_g values were compared to well-matched NDCs, no statistically significant difference was observed ($p = 0.58$ and 0.97 , respectively), demonstrating that PIT corrects insulin resistance in T1D patients despite immunosuppressive therapy.

When islet transplant recipients were divided into groups based on whether they received an islet after kidney transplant or a *de novo* transplant, no difference in insulin sensitivity was noticed between subgroups. In the IAK subgroup, the pre-transplant S_i was $3.8 \pm 0.3 \times 10^{-5}/\text{min}/(\text{pmol/l})$, compared to a pretransplant S_i in ITA of $3.5 \pm 2.1 \times 10^{-5}/\text{min}/(\text{pmol/l})$.

Twelve months post-PIT, the S_i in the IAK group was $6.9 \pm 5.6 \times 10^{-5}/\text{min}/(\text{pmol/l})$, compared to $10.5 \pm 2.6 \times 10^{-5}/\text{min}/(\text{pmol/l})$ for the ITA group (figure 2).

First-phase insulin and C-peptide release after I.V. glucose stimulation are shown in figure 1 (middle panel and insets). Recipient AIR_{GLU} and ACR_{GLU} 12 months after PIT increased to 637 ± 251 pmol/ml·min and 1.6 ± 0.6 nmol/ml·min, respectively, to pretransplant values of 25 ± 61 for AIR_{GLU} and non-detectable levels for ACR_{GLU} ($p = 0.06$ and 0.05 , respectively). However, PIT does not fully restore AIR_{GLU} and ACR_{GLU} to NDC levels (1418 ± 235 pmol/ml·min and 7 ± 1.2 nmol/ml·min; $p = 0.11$ and 0.0001 , respectively).

In addition, DI increased 12 months after PIT (0.058 ± 0.021) compared to pretransplant values (0.0001 ± 0.0002 ; $p = 0.04$); however, DI was statistically significantly lower compared to the NDC group (0.156 ± 0.034 ; $p = 0.04$), likely as a result of insufficient acute insulin response to glucose (Table 3).

Also of interest is the finding that S_g at zero insulin (GEZI), a more precise measurement of insulin-independent glucose disposal than S_g , increases after PIT, approximating the value seen in the NDC group (Table 3).

Significant correlations were established between the K_g Index and S_i ($r = 0.61$, $p = 0.0027$), AIR_{GLU} ($r = 0.44$, $p = 0.02$), ACR_{GTU} ($r = 0.61$, $p = 0.003$) and suprabasal insulin effect DI ($r = 0.59$, $p = 0.0036$) (figure 3). The partial correlation study of different interrelated variables displayed similar correlation coefficients except for GEZI, which seems to be negatively impacted as improvement in glucose disposal is achieved ($r = 0.15$, $p = 0.22$). S_g did not appear to play a significant role in glucose disposal in the study population.

Stepwise multiple regression analysis was performed using the K_g index as the dependent variable and GEZI, S_i , AIR_{GLU} , and DI as independent variables. Only S_i and $S_i \times AIR_{GTU}$ (DI) affected K_g index variation [multiple $r = 0.91$; $r^2 = 0.83$, $p = 0.0001$; model $y = 0.0311(SI) \pm 2.3302(DI) \pm 0.8377$]. S_i alone explained 61% of the K_g index variance, and combined with DI could explain an additional 23% of K_g index recovery. From the partial correlation study, GEZI correlated negatively to correction of K_g values following PIT. Together, these data suggest that correction of S_i and secretion after PIT play a significant role in the improvement of glucose disposal.

Lipid Profile and FFA Clearance Kinetics

Pretransplant cholesterol levels in the 10 islet transplant recipients were 145 ± 33 mg/dl, and at 3, 6 and 12 months post-transplant the values were 169 ± 33 , 176 ± 28 and 167 ± 37 mg/dl, respectively. Triglycerides and HDL pretransplant were 78 ± 53 mg/dl and 53 ± 13 mg/dl, respectively, with mild changes for triglycerides at 3, 6 and 12 months of 89 ± 37 , 101 ± 32 and 113 ± 59 mg/dl and for HDL of 64 ± 12 , 59 ± 9 and 58 ± 8 mg/dl, respectively.

Glucose and insulin values obtained after FSIVGTT pre- and 12 months post-transplant were compared to NDC cohort data (figure 1, bottom panel; Table 4). The FFA profile described by Sumner [21] and for PIT recipients by Rickels et al. [23] is demonstrated in this cohort pre- and post-PIT infusion (Table 4). In parallel with insulin resistance and lack of insulin secretion, baseline FFA values trended higher in the pretransplant population versus post-transplant or NDC (Table 4). Phase 1 summarizes the first 10 min of the FFA profile, demonstrating increased FFA in all groups. Small increments in insulin concentration profoundly affected lipolysis. The FDR for the first 20 min $(FDR)_{0-20}$ after glucose administration reflects the kinetic changes of FFA concentration induced by endogenous insulin release. $(FDR)_{0-20}$ for the pretransplant cohort was $-0.9 \pm 0.7\%/min$ compared to -5.3 ± 2.7 12%/min 12 months post-PIT, presumably because of endogenous increases in AIR_{GLU} and correction of S_i ($p = 0.06$). $(FDR)_{0-20}$ for NDC was $-6.9 \pm 1.6\%/min$, statistically greater only compared to the pretransplant value ($p = 0.0007$). The FDR between 20 and 40 min $(FDR)_{20-40}$ was more pronounced in the pretransplant group versus post-transplant and NDC groups, related to the administration of exogenous insulin at minute 20 after glucose administration. Reduced effect after exogenous insulin administration for PIT and NDC was observed, because endogenous insulin release has already initiated the antilipolytic effect. No differences were noted in terms of FFA nadir or mean FFA in phase 3. Interestingly, during the latter part of the test (phase 3, 80–160 min),

plasma FFA concentrations in the pretransplant cohort were similar to those in the control group and the 12-month post-PIT group (resumption of lipolysis).

Significant correlations were established between the $(FDR)_{10-20}$, S_i ($r = 0.42$, $p = 0.01$) and K_g ($r = 0.32$, $p = 0.05$) almost reaching statistical significance in the suprabasal insulin effect DI ($r = 0.25$; $p = 0.07$) (figure 4), suggesting that changes in glucose disposal strongly induced by changes in S_i and insulin secretion have a direct effect on the observed FFA level decrease (figure 4).

Discussion

This study demonstrates that the main factors determining improvement in I.V. glucose disposal in T1D patients after PIT are improvement in pretransplant insulin resistance, increase in acute insulin response, and insulin-mediated glucose disposal. The highest suprabasal insulin effect observed 12 months post-transplant is associated with the greatest improvement in glucose tolerance, which is directly related to increased AIR_g . FFA dynamics are also improved, and there is a direct increase in S_i and improvement in overall glucose disposal.

Insulin resistance is known to be associated with type 2 diabetes and development of the metabolic syndrome, but its association with T1D is less well documented. Some studies have reported insulin resistance in T1D, typically observed as a transient phenomenon in normal puberty [24] or in patients developing gestational diabetes and later type 2 diabetes [25]. Insulin stimulates glucose transport in adipocytes and skeletal muscle cells by promoting translocation of glucose transporters (GLUT-4) from the intracellular pool to the plasma membranes and by increasing their intrinsic activity [26,27]. The decreased synthesis or decreased intrinsic activity of GLUT-4 in plasma membrane, or both, could explain the decrease in glucose uptake seen pretransplant in T1D with hypoglycaemic unawareness. Suboptimal glycaemic control is also associated with development of insulin resistance in T1D patients [8,28].

Metabolic assays were used in this study to assess post-transplant metabolic function relative to NDC. Comparison between PIT recipients before and after transplant and healthy, well-matched control volunteers provided a standard, allowing qualification of success and failure. The assessment of S_i in T1D subjects utilizing the minimal model approach has been problematic in the past. Diabetic subjects present with a diminished or absent insulin secretory response and insulin resistance. However, modification of the FSIVGTT protocol for diabetic subjects by replacing the standard tolbutamide injection with an injection of insulin allows the calculation of S_i and S_g prior to PIT [29].

Previously published studies suggest that lean T1D patients awaiting islet transplantation suffer from an impaired insulin sensitivity and glucose effectiveness and abnormal free fatty acid dynamics (FFAd) [23]. In addition, using a cross-sectional comparison between two different populations, it was suggested that PIT could potentially correct S_i , S_g and FFAd [23]. In contrast, this manuscript definitively proves for the first time, using a single cohort of patients both pre- and post-PIT, the beneficial effect found after β -cell replacement on the

correction of insulin sensitivity and partial correction of S_g . These findings suggest that factors other than insulin resistance are the obstacles implicated in the short-term duration of the islet allograft. The finding that insulin resistance in T1D patients can be corrected by PIT has important long-term implications, because insulin resistance has been identified as a risk factor for the progression of microvascular (nephropathy, neuropathy and retinopathy) and macrovascular (coronary artery disease and peripheral vascular disease) complications [10,30].

Previous studies after pancreas transplantation have also demonstrated improvement in S_i after re-establishment of β -cell function [31]. In parallel, simultaneous pancreas–kidney transplantation has been shown to normalize hepatic glucose production and reduce peripheral insulin resistance despite chronic immunosuppressive therapy. In contrast to our observation, this study did not demonstrate restoration of S_i to normal values. Steroids [32] and high doses of calcineurin inhibitor [15] have been implicated in the reduction of S_i and impairment in insulin secretion compared to well-matched controls. Elimination of steroid treatment and reduction of calcineurin inhibitors in pancreas transplantation may facilitate a correction of insulin resistance to values similar to nondiabetics and to the ones observed in our islet transplant recipients.

Intensive insulin treatment can also improve S_i in non-transplanted T1D patients [33]. In addition, previously published data suggested that S_i in T1D patients may be partially corrected after PIT even in patients with partial islet function who maintain normoglycaemia using exogenous insulin therapy [34]. In our study, it also appears that the ability of PIT to correct insulin resistance is independent of partial or complete exogenous II. Our results parallel observations by Yki-Jarvinen et al., suggesting that patients with partial β -cell function supplemented with exogenous insulin administration could show improvement in S_i close to normal NDC [35]. Improvement in insulin sensitivity parameters could also be attributed to improvement in glucotoxicity (HbA1c 7.2–5.6%) and lipotoxicity (improved FFA kinetics), which in turn may be because of moderately improved β -cell function. Conversely, correction of HbA1c values after PIT may not be explained exclusively by partial improvement in AIR_{GLU} . The improvement in S_i may be a factor that contributes to their normalization.

Insulin-independent glucose uptake and the effect of hyperglycaemia to suppress hepatic glucose output are determined in the minimal model by the calculation of S_g , which is estimated to account for 20–25% of the K_g variance in healthy individuals [36]. However, it has been shown to be abnormal in type 1 and type 2 diabetics [29,37] and in patients with K_g values $<1.5/\text{min}$ [4]. Our series parallels those findings, because PIT recipients have an abnormal contribution of glucose effectiveness and GEZI to the overall glucose disposal rate. Based on our findings and previously published data, we conclude that although glucose effectiveness is a significant contributing factor to improvement in glucose tolerance, its improvement is not essential to observe an increase in the K_g index [38].

Correction of S_i post-PIT is supported by analysis of FFA disappearance rates after exogenous glucose and insulin administration during modified FSIVGTT. Randle et al. [39] postulate that the ability of insulin to restrain lipolysis is part of its action on glucose

metabolism. By reducing fatty acid substrate availability and through a ‘push’ (membrane transport activation) and ‘pull’ (glycogen synthesis, anaerobic glycolysis and pyruvate oxidation) mechanism [40], insulin promotes glucose transfer. Therefore, abnormalities in glucose and lipid metabolism are expected when insulin resistance is present. It has been suggested that total block of lipolysis is caused almost exclusively by inhibition of hormone-sensitive lipase through endogenous insulin effects, which by itself might explain the differences between pre- and post-transplant FFA suppression. Our finding demonstrating restoration of S_i and K_g values after PIT has significant implications on the FDR of FFA, facilitating the antilipolytic effect on endogenous insulin secretion immediately post-glucose administration. This finding is consistent with data published by Rickels et al., who established a correlation between S_i and glucose tolerance, together with restoration of the capacity to suppress FFA after glucose stimulation in PIT versus T1D patients [23].

Diminished first-phase insulin secretion is recognized as an early marker of β -cell dysfunction, appearing prior to significant changes in glucose concentrations. Subjects with mild β -cell defects (i.e. impaired glucose tolerance) also failed to show restoration of rapid early insulin release despite adequate glucose metabolism once treated with insulin sensitizers. These study results parallel our findings demonstrating that significant improvement in S_i and II after PIT is not associated with full restoration of first-phase insulin release.

Possible pitfalls of the study include the limited number of patients in which observations were available for comparison, as well as possible inclusion of patients with T1D mellitus with hypoglycaemic unawareness who were treated only with intensive insulin therapy as an additional control group. Undoubtedly, the relevance and conclusions of this study will be confirmed by testing a larger population of islet transplant recipients. Additional trials are currently underway.

In conclusion, this study demonstrates that glucose disposal, insulin resistance, S_g and FFA clearance dynamics—measurably abnormal in patients with T1D—can be corrected after PIT. Despite immunosuppression, insulin resistance does not worsen after PIT using a ‘modified Edmonton protocol’. Improvement in glucose disposal in T1D recipients of PIT occurs as a result of improvement in S_i , AIR_{GLU} and DI. In addition, abnormal clearance of FFA after glucose administration can be partially corrected by PIT. As improvements in islet mass engraftment and durability are achieved, better results in terms of FFA suppression will be expected.

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Figure 1.

Frequent sample intravenous glucose tolerance tests performed pretransplant, 12 months post-transplant and in non-diabetic control subjects. (A) Glucose kinetics over 180 min after 300 mg/kg dextrose administration over 1 min starting at $t = 0$. At minute 20, insulin was infused over 30 s at a dose of 0.24 units/kg. (B) Levels of endogenous insulin and C-peptide release (small insert) in addition to the exogenous insulin administered at minute 20. (C) Plasma free fatty acid levels during the FSIVGTT. Data are expressed as mean $\pm$ s.e.

Figure 2.

Insulin sensitivity (A) and glucose effectiveness (B) in islet transplant subjects before and after last islet transplant infusion, compared to non-diabetic control (NDC) subjects.

Pretransplant subjects had statistically significant impairment of insulin sensitivity ($p = 0.0004$) relative to NDCs which is corrected after islet transplantation ($p = 0.003$). Glucose effectiveness is partially corrected after islet transplantation; however, no statistical significance is observed between pre-Tx, post-Tx and NDC.

Figure 3.

Relationship between the K_g index and S_i , S_g , DI , AIR_{GLU} , ACR_{GLU} and glucose effectiveness at zero insulin ($GEZI$) in type 1 diabetic subjects before and after islet transplantation. The solid line depicts the best-fit relationship that was significant for S_i ($r = 0.61$, $p = 0.0027$), DI ($r = 0.59$, $p = 0.0036$), AIR_{GLU} ($r = 0.44$, $p = 0.02$) and ACR_{GLU} ($r = 0.61$, $p = 0.003$), but not for S_g ($r = 0.09$, $p = 0.35$) and $GEZI$ ($r = 0.15$, $p = 0.22$).

Figure 4.

Relationship between the fractional disposal rate (FDR) induced in the first 20 min after intravenous glucose administration and S_I , K_g , AIR_{GLU} and disposition index (DI). The solid line depicts the best-fit relationship that was significant for S_I ($r = 0.42$, $p = 0.01$) and K_g ($r = 0.32$, $p = 0.05$), but not for AIR_{GLU} ($r = 0.11$, $p = 0.24$) or DI ($r = 0.25$, $p = 0.07$). FDR for free fatty acid (FFA) was calculated as the slope of log-transformed FFA values between 10 and 20 min using least-squares linear regression.

Table 1.

Demographic characteristics of 10 islet transplant recipients and their respective controls.

Population	Islet transplant (pretransplant, n = 10)	Non-diabetic control (n = 9)	p
Sex (M/F)	4/6	4/5	
Age (years)	44.2 ± 3.3	46.9 ± 3.5	0.58
BMI (kg/m ²)	23.7 ± 0.6	23.9 ± 0.7	0.82
HbA1c (%)	7.4 ± 0.3	5.6 ± 0.1	<0.001
Fasting glucose (mmol/l)	6.2 ± 0.9	5.4 ± 0.2	0.41
Basal insulin (pmol/l)	22.9 ± 4.6	23.1 ± 4.4	0.98
MAP	91 ± 3	94 ± 3	0.40
Creatinine clearance (ml/min)	91 ± 10	95 ± 6	0.16
Urine protein (g/24 h)	0.19 ± 0.04	NA	NA
Insulin requirement (IU/kg/day)	0.59 ± 0.05	None	

BMI, body mass index; MAP, mean arterial pressure; NA, not available.

Table 2.

Demographic characteristics of 10 islet transplant recipients (5 islet transplant alone and 5 islet after kidney transplants).

Subject no.	Islet alone (ITA) or islet after kidney (IAK)	Immunosuppressive regimen	Number of infusions	Total number of IEQ	Total IEQ/kg	Obtained insulin independence?	Insulin independent at 1 year post-final transplant?	Number of days insulin independent [†]	Units of insulin required (12 months post-initial transplant)	Units of Insulin required (12 months post-final transplant)	Number of severe hypoglycemic episodes at 1 year post-final transplant	HbA1c	1 year post-initial transplant	3 months post-final transplant	6 months post-final transplant	9 months post-final transplant	12 months post-final transplant
1	ITA	Dac, Tac., Sir.	3	1 269 000	20 466	Y	Y	1790	13	0	0	5.9	5.3	5.3	6.7	7.4	ND
2 [*]	ITA	Dac, Tac., MMF	2	834 000	11 440	N	N	0	24	*	*	8.9	*	7.9	*	*	*
3	IAK	Dac, Tac., Sir MMF	3	1 178 659	17 886	Y	Y	372	0	0	0	7.5	4.5	<4	4.4	4.6	4.8
4	ITA	Dac, Tac., Sir	3	2 548 932	31 810	N	N	0	32	25	0	7.4	6.7	5.8	6.8	6.9	6.9
5 ^{**}	ITA	Dac, Tac., Sir	2	2 020 000	27 419	Y	N	270	0	20	0	8	5.6	5.8	5.6	6.5	6.3
6	ITA	Dac, Tac., Sir MMF	2	1 782 111	25 817	Y	Y	1596	0	0	0	7.3	5.6	5.2	5.6	5.1	5.6
7	ITA	Dac, Tac., Sir	3	2 032 850	29 614	Y	N	63	10	15	0	7.1	7.4	7.0	7.4	8	8.4
8	IAK	Dac, Tac., Sir Myfortic	2	1 334 700	25 430	Y	Y	1465	0	0	0	7.7	5.3	6	6.1	5.3	5.3
9	IAK	Dac, Tac., Sir MMF	2	1 061 310	19 187	Y	Y	631	0	0	0	6.7	5.9	5.8	6.0	NA	5.9
10	IAK	Dac, Tac., Sir Tac, Sir M	2	994 350	18 397	Y	Y	521	0	0	0	7.5	5.8	6.1	5.7	5.8	5.6

Patients received an average of 22 746 ± 6289 IEQ/kg body weight. Eight patients achieved insulin independence. Six of 10 patients remained insulin independent > 1 year. Six patients required two infusions and the remaining four patients received three infusions.

^{*} Subject no. 2 withdrew from the study.

^{**} Patient is waiting for third transplant.

[†] As of 2 May 2010.

Table 3.

Metabolic variables pretransplant and 12 months post-islet transplant compared to non-diabetic controls matched by gender, age and BMI.

Measure	Pre-Tx	12 months post-Tx	p (pre vs. post)	NDC	p (pre vs. NDC)	p (post vs. NDC)
BMI (kg/m ²)	23.7 ± 0.7	22.5 ± 0.7	0.29	23.0 ± 1.3	0.65	0.74
Basal glucose (mmol/l)	5.5 ± 0.3	5.3 ± 0.4	0.66	4.5 ± 0.1	0.07	0.21
Basal insulin (pmol/l)	19 ± 9	17 ± 2	0.81	13.3 ± 6	0.67	0.38
K _g	0.96 ± 0.03	1.25 ± 0.09	0.05	1.59 ± 0.15	0.004	0.08
S _i [$\times 10^{-5}$ /min/(pmol/l)]	3.5 ± 0.8	9.6 ± 1.3	0.003	10.8 ± 0.6	0.0004	0.58
S _g ($\times 10^{-2}$ /min)	0.74 ± 0.24	1.28 ± 0.22	0.12	1.27 ± .022	0.21	0.97
BIE ($\times 10^{-2}$ /min)	1.53 ± 0.63	1.69 ± 0.23	0.80	1.41 ± 0.55	0.90	0.59
GEZI ($\times 10^{-2}$ /min)	-0.80 ± 0.75	-0.41 ± 0.26	0.59	-0.14 ± 0.36	0.58	0.60
AIR _g (pmol/l min)	25 ± 61	637 ± 251	0.06	1418 ± 235	0.0001	0.08
ACR _g (nmol/l min)	0 ± 0	1.6 ± 0.6	0.05	7.0 ± 1.2	0.0001	0.001
DI (min)	0.0001 ± 0.0002	0.058 ± 0.021	0.04	0.156 ± 0.034	0.0002	0.04

BIE, basal insulin effect; BMI, body mass index; GEZI, glucose effectiveness at zero insulin; DI, disposition index.

Table 4.

Plasma FFA dynamics during the FSIVGTT.

	Pre-Tx	12 months post-Tx	NDC	p (pre-Tx to NDC)	p (pre- vs. post-Tx)
Baseline (μmol/l)	424 ± 104	245 ± 48	391 ± 64	0.85	0.13
Nadir (μmol/l)	131 ± 37	62 ± 14	102 ± 40	0.66	0.08
AUC insulin phase 1 (pmol/l-min)	25 ± 61	637 ± 251	1418 ± 235	0.0001	0.06
Mean FFA phase 1 (μmol/l)	505 ± 125	333 ± 48	410 ± 104	0.66	0.20
Mean FFA 0–20 min (μmol/l)	500 ± 120	303 ± 42	364 ± 98	0.51	0.13
Fractional disposal rate (%)/min(10–40)	−2.5 ± 0.9	−3.2 ± 1.6	−1.8 ± 2.3	0.73	0.70
Fractional disposal rate (%)/min(10–20)	−0.9 ± 0.7	−5.3 ± 2.7	−6.9 ± 1.6	0.0007	0.06
Fractional disposal rate (%)/min(20–40)	−5.2 ± 1.8	−3.9 ± 2.3	0.1 ± 2.0	0.12	0.67
AUC insulin phase 2 (pmol/l-min)	22 530 ± 5442	11 056 ± 1808	13 162 ± 3742	0.32	0.05
Fractional disposal rate (%)/min(10–70)	−1.9 ± 0.4	−1.4 ± 0.2	−1.0 ± 0.5	0.26	.033
Mean FFA phase 3 (μmol/l-min)	348 ± 43	288 ± 34	335 ± 13	0.85	0.29

AUC, area under the curve; FFA, free fatty acid; Tx, transplant.