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Long term (4 years) improved insulin sensitivity following islet cell transplant in type 1 diabetes

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Abstract

Background—Impaired insulin sensitivity (IS) predicts complications and mortality in type 1 diabetes (T1D). IS improves shortly after islet cell transplant for type 1 diabetes, yet long-term changes in IS, and associated factors such as patient characteristics, transplant factors, clinical management, and IS-related biomarkers are unknown.

Methods—Up to nine years (mean four) of longitudinal data were available on 22 adults (18 female) with T1D who received 1–3 transplants in Phase 1/2 or 3 clinical trials (2004–2014). Metabolic testing post-transplant estimated IS by the Homeostasis Model Assessment for Insulin Resistance (HOMA-IR; 111 observations) and the Simple Index of Insulin Sensitivity (SI_{IS}; 95 observations).

Results—SI_{IS} significantly increased the first year post-transplant ($p=0.02$), then stabilized ($p=0.39$); HOMA-IR remained stable post-transplant ($p=0.92$). Adjusting for age and BMI, higher SI_{IS} was associated with lower HbA1c following transplant ($p=0.03$). Greater IS as measured by lower HOMA-IR and higher SI_{IS} was associated with lower fasting C-peptide (both $p=0.04$), and also with higher exenatide dose (both $p=0.01$). More islets transplanted was associated with higher SI_{IS} ($p<0.0001$). Lower leptin at transplant predicted lower HOMA-IR and higher SI_{IS} after transplant, and lower bone marker RANKL predicted lower HOMA-IR (all $p=0.01$).

Conclusions—IS measured by SI_{IS} was improved several years following transplant, while IS measured by HOMA-IR did not worsen. Higher exenatide dose, more islets transplanted, and diet and exercise (lowering leptin and RANKL) may improve IS, which may enhance glycemic control

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Disclosure Summary

The authors have nothing to disclose.

and lower metabolic demand on transplanted islets. Long-term clamp studies are needed to confirm these results.

Keywords

type 1 diabetes; islet cell transplantation; insulin sensitivity; leptin; receptor activator of nuclear factor kappa-B ligand (RANKL); exenatide

Introduction

Impaired insulin sensitivity (IS) is common not only in type 2 diabetes but also type 1 diabetes (T1D)¹. Tissue uptake of blood glucose is lower than normal, resulting in hyperglycemia despite higher endogenous or exogenous insulin levels, which may lead to complications such as retinopathy, nephropathy, and cardiovascular disease^{2,3}. IS can be quantified using the gold-standard hyperinsulinemic-euglycemic (HIEG) clamp⁴, but the procedure is labor- and time-intensive. Insulin resistance or IS can also be estimated using validated mathematical models such as the Homeostasis Model Assessment for Insulin Resistance (HOMA-IR)⁵ and the Simple Index of Insulin Sensitivity from the oral glucose tolerance test (SI_{is}OGTT)⁶, both highly correlated with HIEG clamp results in nondiabetic individuals^{5–7}. However, as neither HOMA-IR nor SI_{is}OGTT are valid measures in T1D due to the lack of endogenous insulin, an alternative model of IS called the estimated glucose disposal rate (eGDR) was previously derived specifically for patients with T1D⁸. Impaired IS calculated using eGDR has been shown to be clinically significant in T1D as it is strongly associated with longer T1D duration⁹ and predicts diabetes complications and mortality^{10,11}.

To attenuate the burden of hyperglycemia and complications in T1D, islet cell transplantation is a minimally invasive therapy currently being tested in clinical trials as a potential durable treatment for T1D^{12,13}. Briefly, islets are isolated from a deceased human donor pancreas, then transplanted into a T1D patient's liver via the portal vein¹³. The goal is to provide a sufficient number of islets to restore adequate endogenous insulin to control blood glucose and eliminate severe hypoglycemic episodes. However, not all recipients maintain long-term insulin independence, and some experience graft failure¹⁴. Therefore, optimizing recipients' insulin sensitivity could potentially lead to more durable islet graft function by placing less metabolic demand on the transplanted beta-cells. Indeed, research has shown that islet transplant improves IS as measured by the HIEG clamp within 6–7 months following transplant¹⁵.

However, to the best of our knowledge, longitudinal data are lacking describing both long-term changes in IS, and predictors of IS, in patients with T1D who have undergone long-term successful islet transplantation. A better understanding of clinical, modifiable, and/or physiological factors associated with recipients' improved IS could inform future research on interventions aimed at providing more durable islet graft function. Therefore, using longitudinal granular data from patients enrolled in islet transplant clinical trials¹³, the current study: 1) investigated changes in IS using HOMA-IR and SI_{is}OGTT, up to a mean of four years following islet transplant; and 2) determined the longitudinal associations of IS with patient characteristics, transplant factors, clinical management strategies, and select

biomarkers known to be associated with IS in nondiabetic individuals (leptin and adiponectin¹⁶, insulin-like growth factor [IGF-1]¹⁷, and bone-derived markers: receptor activator of nuclear factor kappa-B ligand [RANKL]¹⁸ and osteocalcin [OC]¹⁹).

Materials and Methods

Study Design & Participants

The current study is a secondary analysis of existing data extracted from the medical records of 22 patients with T1D who: 1) were enrolled in the University of Illinois at Chicago's (UIC) islet transplant Phase 1/2 (n=10) or Phase 3 (n=12) clinical trial (NCT00679042) between 2004–2014; and 2) had archived serum tested for biomarkers as part of a prior ancillary study in 2013. The clinical trials have previously been described¹³. Briefly, eligibility criteria included 18–70 years old with T1D for more than five years, experiencing severe hypoglycemic episodes with hypoglycemic unawareness despite optimal therapy. Exclusion criteria included ongoing cardiovascular disease, history of non-skin cancer, other untreated clinically significant comorbidities, previous organ transplant, active smoking, body mass index (BMI) >26 (Phase 1/2) or >27 (Phase 3), insulin requirement $0.7 \text{ U kg}^{-1} \text{ day}^{-1}$, or HbA1c $\geq 12\%$ (108 mmol/mol). Patients received up to three islet transplants to achieve insulin independence. In sum, nine patients received one, six patients received two, and seven patients received three transplants. The first four patients received the Edmonton Protocol for immunosuppression¹²; the remaining 18 received the UIC protocol¹³, which is the Edmonton Protocol plus etanercept and exenatide. Mycophenolate mofetil was substituted for sirolimus if patients presented with intolerable side effects. Management of blood pressure and lipids adhered to the concurrent American Diabetes Association guidelines^{20,21}.

Pre-transplant data used in the current analysis were those closest to the initial transplant. Follow-up after initial islet transplant ranged up to 9.4 years. At the end of 2014, ten of the 22 patients were no longer active in the clinical trials due to: graft failure (n=6), adverse events (n=1), malignancy (n=1), death (n=1), or withdrawal (n=1). However, all of their available data pre- and post-transplant were included in the current analysis. UIC's Institutional Review Board approved the clinical trials and the ancillary study, and written informed consent was obtained.

HOMA-IR and SI_{is}OGTT – Dependent Variables

To calculate HOMA-IR after transplant, fasting glucose and insulin levels were obtained prior to multiple metabolic tests (OGTT, mixed meal test [MMT], intravenous glucose tolerance test [IVGTT], and glucagon stimulation test [GST])⁵. To calculate SI_{is}OGTT after transplant, both fasting and stimulated glucose and insulin levels obtained only from the OGTT were analyzed⁶. Glucose was measured by the glucose oxidase method (UIC Pathology Laboratory, Chicago, IL) and insulin by quantitative chemiluminescent immunoassay (ARUP Laboratories, Salt Lake City, UT). As neither HOMA-IR nor SI_{is}OGTT are valid in T1D, neither was calculated pre-transplant.

OGTT—Glucose and insulin were measured in blood samples taken while fasting, then 30, 60, 90, and 120 minutes after drinking 75gm of glucose (in 300mL solution) within five minutes. This test was scheduled 6, 24, and 52 weeks after each islet transplant, then yearly. Fasting and stimulated levels from 95 OGTTs were used to calculate SI_{is} OGTT, and fasting levels from 47 to calculate HOMA-IR.

MMT, IVGTT, GST—These tests were scheduled 6 (except MMT), 24 and 52 weeks after each islet transplant and every year thereafter. Fasting levels from 18 MMTs, 20 IVGTTs, and 26 GSTs were used to calculate additional HOMA-IR values. The few cases when metabolic testing was also conducted in the days following transplant provided additional data.

Patient Characteristics, Transplant and Clinical Factors – Independent Variables

Patient characteristics included age and duration of T1D at initial transplant, sex, and menopausal status across follow-up. Transplant variables included clinical trial phase, number of transplants, cumulative number of islet equivalents transplanted per kg body weight (IEQs/kg), and immunosuppressant and exenatide doses from each visit. Clinical variables from each visit included BMI, blood pressure after five minutes at rest, insulin dose, and antihypertensive and statin use. Waist and hip circumferences were not part of the clinical trial protocol, so were not available. A comprehensive metabolic profile, which included fasting glucose, was performed at each visit (UIC Pathology Laboratory). Fasting C-peptide was also measured at each visit using quantitative chemiluminescent immunoassay (ARUP Laboratories). Beta-cell function was calculated at the same time points as SI_{is} OGTT as the area under the curve of fasting and stimulated (30, 60, 90, and 120 minute) C-peptide values. Complete C-peptide data were only available in a subgroup of 70 OGTTs. HbA1c was measured every three months by high performance liquid chromatography (UIC Pathology Laboratory). Estimated glomerular filtration rate (eGFR) was calculated using serum creatinine (part of the comprehensive metabolic profile) from each visit (Jaffe reaction, UIC Pathology Laboratory) using the CKD-EPI equation²². An albumin-to-creatinine ratio (Beckman LX20 standard chemistry method, UIC Pathology Laboratory) was obtained every six months. Lipids and inflammatory markers were measured in fasting morning blood samples at pre-transplant, 1, 20, and 52 weeks after each islet transplant, and yearly thereafter (Clinical Reference Laboratory, Lenexa, KS). The lipid panel included LDL, HDL, total cholesterol, triglycerides, and non-esterified fatty acids (NEFAs). Inflammatory markers included plasminogen activator inhibitor-1 (PAI-1) antigen and activity, vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), matrix metalloproteinase-9 (MMP-9), monocyte chemoattractant protein-1 (MCP-1), and high-sensitivity C-reactive protein (CRP). T1D autoantibodies were measured pre-transplant, three times in the first year after initial transplant, and yearly thereafter, and included anti-islet cell (by semi-quantitative indirect fluorescent antibody assay), glutamic acid decarboxylase (by semi-quantitative enzyme-linked immunosorbent assay), IA2 and anti-insulin (both by quantitative radioimmunoassay) (ARUP Laboratories).

Biomarkers – Independent Variables

As part of the clinical trials, serum was archived at -80°C within one hour of the morning fasting blood draw, at three time points: pre-transplant, and 1 and 20 weeks after each islet transplant. As part of an ancillary study in 2013, the archived serum samples from the first transplants only (baseline) were analyzed. Eleven patients had serum available from all three time points, eight from two, and three from one. There were 18 pre-transplant samples (mean [standard deviation (SD)]: 28 [24] weeks prior to first transplant), 20 1-week post-transplant samples (5 [2] days), and 14 20-week post-transplant samples (20 [2] weeks). The following biomarkers were measured: undercarboxylated OC and carboxylated OC by enzyme immunoassay (EIA, Takara Bio, Clontech Laboratories, Mountain View, CA); soluble RANKL by Human sRANKL (TOTAL) ELISA (BioVendor LM, Asheville, NC); IGF-1 by Quantine ELISA Human IGF-1 Immunoassay (R&D Systems, Minneapolis, MN); and leptin and adiponectin by high-sensitivity EIA (R&D Systems). Total OC was calculated by summing the levels of undercarboxylated and carboxylated OC. Patients' mean baseline biomarker levels were calculated using all data available from the three time points.

Statistical Analysis

All analyses were performed using SAS (version 9.4, Cary, NC). A p-value <0.05 was considered statistically significant. The distribution of continuous variables was assessed and transformed if skewed, such as the log transformation of HOMA-IR and $\text{SI}_{\text{isOGTT}}$. Frequencies and percentages were calculated for categorical variables, means and SDs for continuous variables. Histograms and scatter plots identified the following extreme influential outliers that were excluded from the analysis: two log-transformed $\text{SI}_{\text{isOGTT}}$ values, and one baseline mean value each for adiponectin, undercarboxylated OC, and carboxylated OC. For one patient, a single leptin value was excluded and a new mean leptin was calculated.

Longitudinal changes (i.e., the slope) during follow-up in biomarkers, HOMA-IR, $\text{SI}_{\text{isOGTT}}$, and other metabolic outcomes were determined using mixed-effects linear regression modeling of repeated measures. Mixed-effects modeling was used to account for the non-independence of repeated measures within each patient. The autoregressive variance matrix was specified to account for the diminishing within-person correlation of observations that are farther apart in time. To determine the longitudinal association between HOMA-IR and $\text{SI}_{\text{isOGTT}}$, each were standardized by dividing by their respective SD then regressed using the same procedure.

Multivariable mixed-effects linear regression modeling (both forwards and backwards stepwise) was then used to assess for significant associations of the slope of change in HOMA-IR or $\text{SI}_{\text{isOGTT}}$ with the independent variables; both final models were adjusted for age and BMI. Non-linear (i.e., quadratic) relationships of HOMA-IR or $\text{SI}_{\text{isOGTT}}$ with significant biomarkers and continuous independent variables were determined and retained if significant. Effect modification of the associations by patient sex, age, and BMI was also tested and retained if significant.

Results

Patient Characteristics

The majority were female (n=18), and all but one were Non-Hispanic white. The mean (SD) age and duration of T1D at the time of initial transplant were 45.3 (11.9) years and 27.9 (11.8) years, respectively (Table 1). Both pre-transplant and post-transplant, patients were on average normotensive and had normal lipid levels, though many were using anti-hypertensives (n=12 pre/14 post) and/or statins (n=12 pre/17 post). BMI pre- and post-transplant was within the normal range. eGFR levels were, on average, above 80 mL min⁻¹ 1.73(m²)⁻¹ pre- and post-transplant. HbA1c and insulin dose were higher pre-transplant (7.2%, 55.7 mmol/mol; 0.50 U kg⁻¹ day⁻¹) compared to post-transplant (6.2%, 43.8 mmol/mol; 0.10 U kg⁻¹ day⁻¹). The median (25th; 75th percentile) number of IEQs/kg transplanted was 14,309 (7,900; 25,931). The mean (range) follow-up was 4.2 (0–9.4) years.

Biomarkers

Table 2 presents the average levels of each biomarker at the three time points and mean changes over time. There were no significant changes in undercarboxylated OC, carboxylated OC, total OC, or IGF-1. There were trends towards a decrease in RANKL (p=0.09) and an increase in adiponectin (p=0.06). There was a significant decrease in leptin (p<0.0001), specifically from pre- to 1-week post-transplant.

Metabolic Outcomes

Table 3 presents changes in metabolic outcomes: (1) pre-transplant throughout all follow-up, and (2) post-transplant follow-up only. Overall post-transplant, no changes were observed in HOMA-IR or SI_{is}OGTT. HOMA-IR (log) decreased but not significantly within one year after initial transplant (β =−0.12 per 10 weeks, p=0.33; Figure 1a). In contrast, within the first year after initial transplant, there was a significant increase in SI_{is}OGTT (log) (β =0.013 per 10 weeks, p=0.02), which remained stable at this higher level throughout the remainder of follow-up (β =0.001 per 10 weeks, p=0.39; Figure 1b). There was only a moderate, though significant, negative association between HOMA-IR and SI_{is}OGTT (β =−0.33, p=0.002). Fasting glucose and HbA1c decreased significantly from pre- to post-transplant, but only HbA1c decreased significantly during post-transplant only. Fasting C-peptide increased significantly from pre- to post-transplant, but did not change during post-transplant only. No significant changes were observed in fasting insulin or BMI.

Associations with HOMA-IR

Table 4 presents factors associated with HOMA-IR, adjusted for age and BMI. With regard to biomarkers, HOMA-IR after transplant was positively associated with mean leptin at baseline. In addition, mean RANKL at baseline demonstrated a quadratic association with HOMA-IR after transplant (p=0.001; Figure 2a), such that HOMA-IR was slightly negatively associated with RANKL at the lowest levels of RANKL, while positively associated with RANKL at higher levels of RANKL. Fasting C-peptide also had a quadratic association with HOMA-IR (p=0.01; Figure 3a), with a stronger positive association at higher fasting C-peptide levels. Exenatide dose was negatively associated with HOMA-IR in

males, but had a null association in females (sex interaction $p=0.01$). However, female sex had a negative association with HOMA-IR (i.e., they were more insulin sensitive than males, $p=0.001$). Further adjusting for the type of metabolic test used to calculate HOMA-IR did not change the results.

Associations with SI_{is} OGTT

Adjusting for age and BMI (Table 4), mean leptin at baseline demonstrated a quadratic association with SI_{is} OGTT post-transplant ($p=0.01$; Figure 2b), with the negative association being stronger at lower leptin levels, and the association plateauing at higher leptin levels. Fasting C-peptide also demonstrated a quadratic association with SI_{is} OGTT, but in opposite directions from the HOMA-IR association ($p=0.04$; Figure 3b), with a stronger negative association at higher fasting C-peptide. In a smaller number of observations ($n=59$), the C-peptide area under the curve from the OGTT replaced fasting C-peptide and demonstrated a negative, but linear, association with SI_{is} OGTT (log) ($\beta=-0.004$, $p=0.003$). SI_{is} OGTT was positively associated with exenatide dose and cumulative IEQs/kg transplanted, while negatively associated with HbA1c. Cumulative IEQs unadjusted for recipient weight was interchangeable with IEQs/kg in the model. Pre-menopausal women were more sensitive to insulin than men, while post-menopausal women and men had similar SI_{is} OGTT.

Variables not associated with either HOMA-IR or SI_{is} OGTT were: duration of T1D, blood pressure, lipids and NEFAs, inflammatory markers, statin and antihypertensive use, insulin dose post-transplant, immunosuppression, eGFR, albumin-to-creatinine ratio, T1D auto-antibodies, OC, adiponectin, and IGF-1. Further, adjusting all the final models for the minimal insulin dose patients may have still required immediately post-transplant did not substantially change the results for either HOMA-IR or SI_{is} OGTT.

Discussion

To the best of our knowledge, this is the first study to investigate long-term changes in IS using HOMA-IR and SI_{is} OGTT in patients with T1D who have received islet cell transplantation (even if requiring small doses of exogenous insulin immediately after the transplants); and to determine clinical, modifiable, and/or physiological factors associated with long-term changes in IS following islet transplant in these patients. In summary, IS as measured by SI_{is} OGTT improved within one year following first transplant, and remained stable at this improved level for up to a mean of four years, whereas IS as measured by HOMA-IR remained stable (i.e., did not worsen) over the entire follow-up. This is particularly important given that decreasing IS is associated with longer T1D duration⁹. IS after transplant was associated with both potentially modifiable factors and with transplant outcomes. These results need to be confirmed using HIEG clamp studies in the decade following islet transplant, but indicate potential clinical benefits of optimizing IS surrounding transplant.

Our study is in agreement with prior research that also demonstrated IS (peripheral, hepatic, and total) improved immediately post-islet transplant, as quantified by the FSIVGT²³ and the HIEG clamp¹⁵, however both of these studies were limited to at most one year follow-up. Research in a more heterogeneous sample, where 4 of the 10 participants were islet-

after-kidney recipients, similarly found improvements in IS measured by FSIVGT within 12 months following islet transplant²⁴. The beneficial effect that islet transplant has on IS has been hypothesized to be a result of restoring endogenous insulin, specifically the resolution of hyperglycemia and normalization of insulin's suppression of NEFA production²³. The current study interestingly found a significant improvement in IS as measured by SI_{is}OGTT, but no change in IS as measured by HOMA-IR. Further mechanistic research is needed to determine if islet transplant has differential effects on parameters quantified by SI_{is}OGTT's stimulated metabolic outcomes, specifically muscle and adipose tissue glucose metabolism, versus parameters captured by HOMA-IR, specifically fasting hepatic glucose production^{6,25}. Or perhaps SI_{is}OGTT may be a more sensitive measure of insulin action in this population.

With respect to clinical outcomes, as expected, HbA1c and fasting C-peptide improved after transplant²⁶. Among individuals with T1D, previous research has shown that higher IS (HIEG clamp) was associated with lower glycosylated hemoglobin²⁷. The current study similarly found that for patients undergoing islet transplant for T1D, enhanced IS following islet transplant was associated with improved glycemic control. Lower IS was significantly associated with greater fasting C-peptide, and in a smaller subgroup, with greater stimulated C-peptide as well. In this case, C-peptide, a marker of endogenous insulin secretion, might therefore also be interpreted as a marker for beta-cell demand or stress, since it is generally accepted that impaired IS requires higher levels of insulin, increasing demand on beta-cells²⁸. The quadratic association between fasting C-peptide and IS as measured by both HOMA-IR and SI_{is}OGTT (Figure 3) demonstrates that the positive and negative associations, respectively, are stronger above the normal value of fasting C-peptide, about 1.0 ng/mL, such that it is the patients who are less sensitive to insulin that are placing more demand on their islet grafts' beta-cells. Therefore, improving patients' IS surrounding islet transplant could potentially lower the demand on beta-cells, extending islet graft function and survival.

Other islet transplant trials have shown that in patients receiving more than 600,000 IEQs in total, 75–80% reached insulin independence, compared with 55% who received fewer²⁹. The current study found that having more IEQs/kg transplanted was also associated with improved IS. This may be a function of a greater number of IEQs providing a more rapid resolution of hyperglycemia and suppression of NEFA production as previously discussed²³. Also demonstrated was a sex difference in the relationship of exenatide with IS as calculated by HOMA-IR, such that exenatide was associated with improved IS among men, but not in women. In comparison, exenatide was associated with improved IS as calculated by SI_{is}OGTT in both sexes. Exenatide has been previously shown to improve IS (HIEG clamp) by 40% in patients with T1D³⁰. The lesser impact of exenatide specifically on IS in women following islet transplant may be a function of their already significantly higher IS compared to the men in the present sample.

The current study is innovative in that it investigated the association of IS post-transplant with IS-related biomarkers measured at baseline. The demonstrated association between HOMA-IR and RANKL (specifically at higher levels of RANKL) is similar to the findings of an experimental study whereby the blocking or deletion of RANKL signaling specifically

in the liver in mice led to significantly lower HOMA-IR compared to wild type mice on a high fat diet¹⁸. In an epidemiological study, higher baseline circulating levels of RANKL were found to be associated with greater odds of developing type 2 diabetes over a 15 year period¹⁸. Further research is needed to determine if the quadratic association between HOMA-IR and RANKL (Figure 2a) reflects various thresholds where there is an optimal circulating range of RANKL, above and below which IS decreases. Regarding leptin, similar to the current study, previous literature found a positive association between leptin and HOMA-IR among nondiabetic individuals¹⁶. The current study also found a negative, quadratic association between leptin and SI_{is} OGTT (Figure 2b). Again, further research is needed to clarify if the association is capturing a threshold effect where below a certain leptin concentration, the negative association of leptin with IS is stronger.

Based on our findings, islet transplant outcomes may be optimized by considering both transplant and patient modifiable factors that enhance patients' IS. Maximizing the number of IEQs transplanted in the patient's first transplant may result in better IS, which then may lead to lower fasting C-peptide and better glycemic control as found in this study, as well as potentially longer lasting graft function. Prescribing and titrating exenatide to the maximum tolerable dose may be an option for improving IS after transplant, and potentially pre-transplant. Another option for enhancing patients' long-term IS after transplant may be lowering leptin and RANKL levels surrounding first transplant by prescription of healthy diet³¹ and exercise^{31,32}.

Although this study does not utilize the gold standard for measuring IS (HIEG was not part of the clinical trial protocols), the employed metrics, HOMA-IR and SI_{is} OGTT, have been documented to be highly correlated with HIEG clamp measures in nondiabetic, non-obese individuals^{5,7}. The trends in, and associations with, HOMA-IR and SI_{is} OGTT remained robust even after adjusting for the minimal exogenous insulin therapy patients were requiring shortly after the islet transplants, supporting the use of these mathematical models in this patient population after T1D was reversed. Just as HOMA and SI_{is} OGTT were not calculated pre-transplant as they are not valid in the presence of T1D, eGDR was not calculated post-transplant as it was specifically derived and validated for use in those with T1D⁸. As a standard IVGTT (not a FSIVGT) was part of the clinical trial protocols, with fewer blood samples and without a bolus of insulin, we were not able to calculate the Insulin Sensitivity Index using minimal modeling. As is the case for *all* islet transplant clinical trials, a control group was not available, because it is unethical to randomize patients to either islet transplant or an alternative treatment for hypoglycemic unawareness given that in order to be eligible for islet transplantation, the patients would have already failed such existing treatments. Furthermore, patients cannot serve as their own control because neither HOMA-IR nor SI_{is} OGTT can be calculated pre-transplant as these measures are not valid in patients with T1D. However, looking at existing controls in the literature, it has been demonstrated that IS decreases with longer duration of diabetes in those living with T1D⁹, indicating the improvement in SI_{is} OGTT and the stabilization of HOMA-IR after transplant may be clinically important. The sample had limited diversity, with mostly Non-Hispanic white females, limiting the generalizability of these findings. Because the primary endpoints for islet transplant clinical trials are safety and efficacy, diet and exercise data were not obtained. A strength of this study was its use of longitudinal data, with a mean of four years

of follow-up. With the smaller sample size, all available data were able to be analyzed despite unequal follow-up times using the mixed-effects modeling of repeated measures.

In summary, IS as measured by SI_{is} OGTT improved within one year of functionally curing T1D via islet transplant and remained improved, while IS as measured by HOMA-IR did not worsen, over several years of follow-up. Furthermore, improved IS was associated with better glycemic control and lower fasting C-peptide. However, these results need to be confirmed using HIEG clamp studies over the decade following islet cell transplant. With the long-term goal being to lessen the requirement of islets transplanted along with the need for subsequent islet transplants, utilizing clinical and behavioral modifications pre- and post-transplant to improve patients' IS may be considered. Future research is needed to confirm the clinical significance of such interventions on subsequent clinical outcomes in this patient population.

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Figure 1. Individual patient change in a) HOMA-IR (inset log-transformed), and b) $SI_{is}OGTT$ (inset log-transformed) during follow-up post-transplant. Dashed line indicates 1 year follow-up.

Figure 2.
Nonlinear associations between a) HOMA-IR post-transplant and RANKL at baseline, and
b) SI_{is}OGTT post-transplant and leptin at baseline

Figure 3.
Nonlinear associations between a) HOMA-IR and fasting C-peptide post-transplant, and b) SIIsOGTT and fasting C-peptide post-transplant

Table 1

Characteristics of Study Sample (n=22)

Characteristic	Pre-Transplant		Post-Transplant	
Age (years), mean (SD)	45.3	(11.9)		
Female sex, n (%)	18	(82)		
Pre-menopausal, n (%)	10	(56)	7	(39)
BMI (kg/m ²), mean (SD)	23.3	(1.9)	22.1	(1.9)
Duration of T1D (years), mean (SD)	27.9	(11.8)		
HbA1c, mean (SD) ^a				
%	7.2	(0.9)	6.2	(0.7)
mmol/mol	55.7	(9.7)	43.8	(7.5)
Insulin dose (units/kg), mean (SD)	0.50	(0.16)	0.10	(0.16)
Blood pressure (mmHg), mean (SD)				
Systolic	115.8	(12.8)	125.8	(15.8)
Diastolic	67.3	(8.0)	72.8	(9.3)
Antihypertensive use, n (%)	12	(55)	14	(64)
Cholesterol (mg/dL), mean (SD)				
Total	164.8	(34.7)	182.1	(43.4)
HDL	64.5	(19.7)	64.7	(26.1)
LDL	85.5	(27.5)	98.1	(32.1)
Triglycerides	76.5	(60.5)	94.5	(45.4)
Statin use, n (%)	12	(55)	17	(77)
eGFR (mL min ⁻¹ 1.73(m ²) ⁻¹), mean (SD)	82.2	(20.1)	81.1	(22.1)
Number of transplants received, n (%)				
1			9	(41)
2			6	(27)
3			7	(32)
Total IEQs/kg, median (25 th ; 75 th percentile)			14,309	(7,900; 25,931)
Protocol, n (%)				
Edmonton			4	(18)
UIC			18	(82)
Phase, n (%)				
1/2			10	(45)
3			12	(55)
Follow-up time (years), mean (SD)			4.2	(2.9)

Pre=calculated using closest value prior to first transplant; Post=calculated using all data available post-first transplant

^aHbA1c pre-transplant, n = 21

Table 2

Changes in Biomarker Levels During Follow-up (22 patients with 52 observations)

Biomarker, mean (SD)	Pre-Transplant n=18	1 week Post n=20	20 weeks Post n=14	β per 10 weeks (SE)	p-value
Undercarboxylated OC (ng/mL)	6.1 (5.0)	5.5 (2.9)	3.9 (6.0)	-0.10 (0.14)	0.50
Carboxylated OC (ng/mL)	23.5 (14.0)	21.9 (14.8)	24.1 (12.6)	0.16 (0.39)	0.69
Total OC (ng/mL)	29.6 (14.4)	27.4 (14.8)	28.0 (13.3)	0.06 (0.41)	0.88
RANKL (pmol/L)	4.0 (5.2)	4.1 (4.4)	3.4 (3.4)	-0.24 (0.13)	0.09
IGF-1 (ng/mL)	98.6 (38.4)	95.2 (33.9)	96.1 (34.8)	-0.40 (1.20)	0.74
Leptin (ng/mL)	43.2 (23.3)	19.3 (10.4)	19.4 (10.5)	-4.34 (0.83)	<0.0001
Adiponectin (ng/mL)	7464 (3700)	8483 (3370)	9012 (3140)	254 (127)	0.06

Table 3

Changes in IS and Other Metabolic Outcomes During Follow-up (22 patients)

Outcome	Pre- thru Post-Transplant			Post-Transplant Only		
	β per 10 weeks (SE)	n	p-value	β per 10 weeks (SE)	n	p-value
HOMA-IR (log)	–	–	–	0.0009 (0.009)	111	0.92
SI ₁ OGTT (log)	–	–	–	0.0003 (0.001)	95	0.71
Fasting glucose (mg/dL; log)	–0.006 (0.002)	151	0.01	–0.002 (0.002)	130	0.48
Fasting C-peptide (ng/mL)	0.017 (0.006)	151	0.003	0.004 (0.006)	130	0.47
Fasting insulin (μ U/mL; log)	0.0004 (0.008)	117	0.96	0.001 (0.008)	111	0.92
HbA1c						
%	–0.03 (0.01)	138	0.0006	–0.01 (0.01)	117	0.04
mmol/mol	–0.27 (0.08)	138	0.0006	–0.15 (0.07)	117	0.04
BMI (kg/m ²)	–0.01 (0.02)	147	0.43	0.01 (0.02)	126	0.78

n=number of longitudinal observations

Table 4Factors Associated with HOMA-IR and SI_{is}OGTT (adjusted for age and BMI; n=22)

Independent Variable	HOMA-IR (log) 107 observations			SI _{is} OGTT (log) 85 observations		
	β	(SE)	p-value	β	(SE)	p-value
Intercept	3.30	(0.89)	0.002	-1.30	(0.09)	<0.0001
Mean leptin (ng/mL)	0.049	(0.008)	<0.0001	-0.009	(0.002)	0.001
Mean leptin x mean leptin				0.0001	(0.00004)	0.01
Mean RANKL (pmol/L)	-0.34	(0.09)	0.002			
Mean RANKL x mean RANKL	0.025	(0.006)	0.001			
Sex (male=0, female=1)	-1.14	(0.29)	0.001			
Fasting C-peptide (ng/mL)	-0.77	(0.37)	0.04	0.04	(0.03)	0.23
Fasting C-peptide x fasting C-peptide	0.35	(0.13)	0.01	-0.02	(0.01)	0.04
HbA1c (%)				-0.02	(0.01)	0.03
Exenatide dose (μ g)	-0.12	(0.04)	0.01	0.004	(0.001)	0.0001
Exenatide dose x sex	0.12	(0.05)	0.01			
Cumulative IEQs/kg (per 1000)				0.003	(0.001)	<0.0001
Pre-menopausal women vs. men				0.05	(0.02)	0.03
Post-menopausal women vs. men				-0.02	(0.02)	0.43