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Pretransplant HOMA-β Is Predictive of Insulin Independence in 7 Patients With Chronic Pancreatitis Undergoing Islet Autotransplantation

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Background. Islet and β-cell function is intrinsic to glucose homeostasis. Pancreatectomy and islet autotransplantation (PIAT) for chronic pancreatitis (CP) treatment is a useful model for assessing islet function in the absence of immune-suppression and to perform extensive presurgical metabolic evaluations not possible from deceased donors. We recently showed that in CP-PIAT patients, preoperative islet identity loss presented with postoperative glycemic loss. Here, we examine presurgical islet function using Homeostatic Model Assessment-Beta Cell Function (%) (HOMA-β) and glycemic variables and compared them with postsurgical insulin independence and their predicted alignment with Secretory Unit of Islet Transplant Objects (SUITO) and beta cell score after transplantation (BETA-2) scores. **Methods.** Seven CP-PIAT patients were assessed for β-cell function metrics, including pretransplant and 6-mo posttransplant HOMA-β using insulin and C-peptide and evaluations of proposed insulin independence by SUITO and BETA-2 graft function equations. These were compared with oral glucose tolerance tests and pancreas histological samples taken at the time of transplant, examined for β-cell maturity markers. **Results.** Pre-PIAT, HOMA-β (60%–100%) associated with post-PIAT insulin independence. This association was only moderately supported by post-PIAT SUITO threshold scores (≥26) but robustly by BETA-2 scores (≥16.2). Appropriate posttransplant oral glucose tolerance test curves were found in those patients with normal pretransplant HOMA-β values. Preoperative low serological β-cell function was displayed by concurrent evidence of β-cell identity alterations including colocalization of insulin and glucagon, loss of urocortin-3, and increased intra-islet vimentin in patients who were insulin-dependent post-PIAT. **Conclusions.** These data encourage HOMA-β assessment before PIAT for estimating posttransplant insulin independence.

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Islet and β-cell function are critically important for glycemic homeostasis. Loss of islet function has been linked to loss of islet identity, resulting from α- (alpha) and β- (beta) cell dedifferentiation from chronic overnutrition or cellular stress,[1] and may be visualized by nonbinary endocrine hormone presence,[2-4] loss of maturity markers V-maf musculoaponeurotic fibrosarcoma oncogene homolog A (MAFA)[5] or urocortin-3 (UCN3),[6] and/or gain of intra-islet mesenchymal protein vimentin (VIM),[4,7] independent of the fibrosis found in chronic pancreatitis (CP) pancreatic acini. Metabolic stress can reduce islet size, islet area, and β-cell mass, the latter being a defining characteristic of all diabetes subtypes,[8] which underlies the eventual loss in insulin secretion and consequent hyperglycemia. Pancreatectomy and islet autotransplantation (PIAT) for the treatment of CP is a useful model for assessing islet function in the absence of immune-suppression and to perform extensive presurgical metabolic evaluation not possible in deceased-donor-sourced donor islet transplants. The progressive inflammation and associated fibrosis in CP has been shown to cause endocrine insufficiency and eventual diabetes, with significant islet damage.[9] Indeed, although it is logical and expected that insufficient transplanted islet mass has poorer glycemic outcomes,[10,11] achieving or even surpassing suggested transplanted islet number alone is still inadequate to guarantee insulin independence, and the role and

methods of defining pretransplant islet function are critically required. We recently showed that CP itself contributes to islet identity loss[12] and is an independent variable from the clinical guideline of transplanting ≥5000 islet equivalents (IE) per kilogram body weight.[13,14] These results demonstrated that islet and β-cell–specific loss of identity was not exclusively related to pretransplant glucose levels, as all patients were nondiabetic at the time of surgery. Furthermore, although islet identity loss was associated with pretransplant elevated insulin resistance (IR) metrics including fasting proinsulin, insulin, proinsulin/insulin ratio, and Homeostatic Model Assessment-Insulin Resistance (HOMA-IR), no patient demonstrated extant diabetes. Instead, data suggested dynamic alterations in islet and β-cell function.

Here, we examined 7 PIAT patients and compared pretransplant serum glycemic variables including Homeostatic Model Assessment-Beta Cell Function (%) (HOMA-β), a marker of β-cell function using fasting glucose and insulin[15,16] or C-peptide,[17] to 6-mo postoperative insulin independence. Posttransplant, we utilized 2 scoring tests to assess proposed versus actual insulin independence :(1) the Secretory Unit of Islet Transplant Objects (SUITO) index,[18] which uses C-peptide posttransplant in type 1 diabetes (T1D) patients taking insulin, and (2) the beta cell score after transplantation (BETA-2) score,[19,20] which encompasses diverse variables to assess graft function, including insulin dose. All patient pancreas biopsies at PIAT were examined histologically for changes in β-cell identity. We hypothesized that reduced β-cell function, as revealed by abnormal pretransplant HOMA-β values, would correlate with insulin dependence post-PIAT, and consequently, subthreshold BETA-2 and SUITO scores for insulin independence. Results demonstrate that noninvasive pretransplant HOMA-β may estimate insulin independence following PIAT.

MATERIALS AND METHODS

Patient Clinical Data

Ethical approval and informed consent for research were acquired from patients before surgery in this single-center, retrospective analysis. All PIAT procedures occurred at Houston Methodist Hospital, Houston, TX (IRB 0907-0178) between June 2008 and October 2010. Following surgical board review and insurance approval, a total of 9 patients were enrolled in the trial during this period (2 male/7 female). Both male participants were later excluded from study analysis, one lost from follow-up >2 mo, and the other because of incomplete presurgical glycemic testing. The remaining 7 PIAT patients completed preoperative glycemic testing in hospital by commercial laboratory methods using venous blood, and did not have diabetes by American Diabetes Association (ADA) criteria including hemoglobin A1c (HbA1c) ≤6.5%, fasting glucose ≤126 mg/dL (7.0 mmol/L), and/or 2 h glucose on oral glucose tolerance tests (OGTTs) ≤200 mg/dL (11.1 mmol/L) with 75 g glucose ingestion.[21] OGTT was completed after a 12 h fast, with blood sampling at 0, 30, 60, 90, and 120 min. Fasting insulin (3–8 uIU/mL), proinsulin (2.5–8.8 pmol/L), and C-peptide (0.8–1.9 ng/mL) were measured as previously reported,[12] with glucose tested by automated chemistry analysis, C-peptide assayed by quantitative electrochemiluminescent immunoassay, and insulin and proinsulin assayed by quantitative chemiluminescent immunoassay. Reports suggest proinsulin >10 pmol/L is a marker of IR,[22] the proinsulin-to-insulin ratio >0.3 is a marker of β-cell distress independent of IR,[23] and elevated proinsulin-to-C-peptide ratio (PI/C) may be a superior marker of hyperproinsulinemia than insulin in predicting diabetes.[24] Islets were isolated as described.[25] Patient demographics, pancreas weight, and IE/kg are reported in Table 1. OGTT/glucose-stimulated insulin secretion (GSIS) were repeated at 6 and 12 mo post-PIAT.

Estimations of Endocrine Function and Insulin Independence

IR and β-cell function scores were calculated using Homeostatic Model Assessment (HOMA) from fasting plasma insulin (FPI) (μIU/mL) and fasting plasma glucose (FPG) (ng/mL), using the equations HOMA-IR = (FPI × FPG)/405 and HOMA-β = (360 × FPI)/(FPG−63)(%).[15,16] HOMA-β (C-Pep) alternatively was determined using fasting C-peptide (FCP) by (0.27 × FCP)/(FPG−3.5) because of the post-PIAT exogenous insulin requirement in most patients. HOMA-β values additionally were determined from the computer program/calculator (v.2.2.3, Diabetes Trials Unit, Oxford University, United Kingdom),[15] as the calculator precludes insulin values <2.9 μU/mL and C-peptide values <0.6 ng/mL. IR was defined as HOMA-IR >2.[26] Normal β-cell function has HOMA-β scores 60%–100%[27]; scores <40 are considered clinically dysfunctional,[18] and those >100% indicate overwork.[28] The SUITO index determines endogenous insulin secretion in T1D patients requiring insulin injection, by FCP (ng/mL)/(FPG−63 [mg/dL]) × 1500, with values ≥26 reportedly correlating with insulin independence.[18] The BETA-2 score[19] is updated from the BETA score,[29] quantitated by ((√FCP (nmol/L) × (1−total daily insulin dose units/kg))/((FPG (mmol/L) x HbA1c (%)) within the range of 0–42; scores ≥16.2 reportedly correlate with insulin independence in T1D[19] and PIAT[20] patients.

Immunofluorescence and Histomorphometric Analysis

Immunofluorescent histology was performed as described from a single pancreas biopsy (1 cm^2, head) at PIAT.[12] Briefly, 5-μm paraffin-embedded sections were immunostained following SNIPER block (Biocare Medical, Concord, MA). Primary antibodies including rabbit anti-insulin (1:200; Santa Cruz Biotechnology, Dallas, TX), mouse anti-insulin (1:2000; Sigma, St. Louis, MO), rabbit anti-VIM (1:250; Abcam), mouse anti-glucagon (1:2000; Sigma), mouse anti-synaptophysin (1:50, BD Biosciences), or rabbit anti-urocortin 3 (a kind gift from Dr Paul Sawchenko) were incubated overnight at 4 °C. Fluorescent secondary antibodies conjugated to 488-and 555-nm fluorophores (1:500; Alexa Fluor, Invitrogen) were incubated in the dark for 2 h. Cell nuclei were counterstained using DAPI (4, 6-diamidino-2 phenylindole, dihydrochloride). Every endocrine-expressing cell was imaged using a Nikon A1 confocal microscope, analyzed using ImageJ software (v.1.50i, NIH, Bethesda, MD) and counted manually per section.

Islet and β-cell area and β-cell mass were assessed from each pancreas sample,[30] immunostained for insulin and synaptophysin and traced at ×20 magnification (Intuos, Wacom Technology Corporation, Vancouver, WA), and pancreas tissue area by DAPI at ×10. The relative area was quantified using Image J, stitched together by Image Composite Editor (Microsoft Research). Percent beta-cell area (μm^2) was calculated by dividing the % insulin-immunostained area by total

TABLE 1.

Patient demographics, fasting glycemic serology, calculations of HOMA-IR and HOMA-β using insulin and C-peptide, and comparison with predictions of insulin independence by the SUITO and BETA-2 equations in 6 patients with chronic pancreatitis before and 6 mo after undergoing total pancreatectomy and islet autotransplantation

Characteristic	P1	P2	P3	P5	P6	P7	*P*
Patient demographics							
Sex	Female	Female	Female	Female	Female	Female	NA
BMI	25.7/23.5	26.2/21.6	23.1/23.2	23.6/24.9	20.5/18.6	18.3/18.3	NA
Age	44	34	59	65	52	49	0.0833
Race/ethnicity	White	White	White	White	White	White	NA
CP etiology	Pancreas divisum	Sphincter of Oddi dysfunction	Idiopathic	Idiopathic	Idiopathic	Idiopathic	
Disease duration (y)	2	5	10	4	4	8	1.000
Pancreas weight (g), PIAT	32.2, Com[a,b]	85.6, Tot[c]	58.1, Tot	26.6, Com[a,b]	36, Sub[d,f]	74.7, Tot[e]	NA
IE/kg	4610	4907	4916	1716	620	6723	1.000
Insulin dose (U/kg)	0	0.29	0.29	0.4	0.26	0	NA
Fasting serology (pre-/post-PIAT)							
Glucose (mg/dL)	89/85	85/157	91/131	94/140	104/102	73/94	0.0833
C-peptide (ng/mL)	1.3/1.2	1.6/0.9	NP/1.9	2.4/0.8	0.9/0.8	1.3/1.0	0.3613
Insulin (ulU/mL)	7/5	8/11[g]	11/9[g]	2/19[g]	3/3[g]	5/9	0.0833
Proinsulin (pmol/L)	5.3/8.0	25.9/45.7	5.6/11.3	NP/10	4.9/3.5	2.2/8.6	0.2207
PI/C-pep ($pmol^{-1}$)	1.22/2.0	4.86/15.3	X/1.77	X/3.76	1.63/1.32	0.51/2.58	**0.0253**
PI/Ins ($pmol^{-1}$)	0.11/0.23	0.46/0.59	0.07/0.18	X/0.08	1.63/0.17	0.06/0.14	0.1380
HbA1c (%)	5.4/6.1	4.9/6.5	4.9/6.3	4.4/6.9	6.0/5.9	5.5/6.5	0.4386
HOMA values (pre-/post-PIAT)							
HOMA-IR (Ins) (eq)	1.5/1.1	1.7/4.3	2.5/2.9	0.5/6.6	0.8/0.8	1.1/2.0	0.4386
HOMA-β (Ins) (eq)	96.9/81.8	130.9/42.1	141.4/47.6	23.2/88.9	26.3/27.7	78.3/105	**0.0143**
HOMA-β (Ins) (calc)	91.4/80.0	109.7/41.5	118.7/58.4	X/76.5	37.8/39.3	78.1/97.2	**0.0253**
HOMA-β (C-pep) (eq)	80.9/88.3	117.7/15.5	NP/45.2	125.3/16.8	35.5/33.2	91.5/52.2	**0.0253**
HOMA-β (C-pep) (calc)	94.3/97.7	118.8/24.7	NP/58.9	129.5/58.2	54.1/51.9	100.8/70.8	**0.0253**
Islet graft function equations for insulin independence (post-PIAT)							
SUITO index	81.8	14.4	41.9	15.6	30.8	48.4	0.2207
BETA-2 score	22.1	6.7	12.3	9.6	12.7	17.4	**0.0143**
Insulin independent	Yes	No	No	No	No	Yes	

Previous pancreatic surgery:
[a] Whipple,
[b] cholecystectomy,
[c] sphincteroplasty for sphincter of Oddi dysfunction,
[d] ERCP,
[e] Puestow (pancreaticojejunostomy),
[f] pancreatic ductal stent placement.
[g] Insulin use posttransplant.

HOMA data shown represent values pre/6 mo postsurgery. *P* calculated using pretransplant values as noted, by χ^2 with significance set at $P < 0.05$, shown in bold. Chi-squared (χ^2) assessment was compared against normal values/ranges as stated in text, or median patient values as follows: age (<51 y); fasting glucose (<90 mg/dL); IE/kg (>4758); PI/C ratio (<1.43); disease duration (>4.5 y); and HbA1c (≤5.7%).

BETA-2, beta cell score after transplantation; BMI, body mass index; cal, calculator; Com, completion pancreatectomy; CP, chronic pancreatitis; C-pep, C-peptide; eq, equation; ERCP, endoscopic retrograde cholangiopancreatography; HbA1c, hemoglobin A1c; HOMA-IR, Homeostatic Model Assessment-Insulin Resistance; HOMA-β, Homeostatic Model Assessment-Beta Cell Function (%); IE, islet equivalents; Ins, insulin; NA, not available; NP, value not provided; PI/C, proinsulin-to-C-peptide ratio; PI/Ins, proinsulin-to-insulin ratio; PIAT, pancreatectomy and islet autotransplantation; Sub, subtotal pancreatectomy; SUITO, Secretory Unit of Islet Transplant Objects; Tot, total pancreatectomy; X, value too low for use by calculator/computer program.

pancreas area. β-cell mass (g) was calculated by multiplying β-cell area by pancreas weight.

Statistical Analysis

Significant differences between preoperative glycemic variables were calculated using chi-squared (χ^2) test versus 6-mo insulin independence using normal ranges or median patient values[31] as noted (Table 1). SUITO sensitivity/specificity was assessed versus BETA-2 by receiver operating characteristic curve. OGTT were expressed as mean ± SEM, and area under the curve (AUC) analyzed by unpaired, 2-tailed Student *t* test from OGTT insulin and glucose curve totals. Histological data were expressed as %mean ± SEM of all β-cells per section counted, analyzed by unpaired *t* test with Welch correction between groups. Analysis was performed using GraphPad Prism software (v6, GraphPad, San Diego, CA), and with a significance of $P < 0.05$.

RESULTS

Insulin-independent Patients After Islet Autotransplant Demonstrate Preserved, and Calculated, β-cell Function

All patients (n = 7) were female, aged 52.1 ± 9.8 y, non-Hispanic White race/ethnicity, body mass index 23.3 ± 2.9 kg/m^2, and 6 of 7 had previously undergone ≥1 pancreatic surgery before pancreatectomy (Table 1). Six of 7 patients had islets transplanted into the portal system and 1 of 7 into the arm[32]; this patient (P4) was thereafter excluded from serology grouped analysis but is presented in supplemental material for comparison. Younger age (<51 y), but not disease duration (>4.5 y), nor IE/kg (>4758), trended with insulin independence ($P < 0.1$; Table 1) in the 6 grouped patients.

Preoperatively, 1 of 6 patients demonstrated impaired fasting glucose and mildly elevated HbA1c (P6; Table 1).

Six months after PIAT, 2 patients were insulin independent (P1 and P7); both exhibited presurgical normal fasting glucose and C-peptide, and HbA1C ≤6.5% (Table 1). The remaining 4 patients required supplemental insulin. FCP was elevated in P5 before surgery (Table 1). Following PIAT, there were elevations in fasting glucose in most insulin-dependent patients (P2–P5; Table 1). Pretransplant fasting insulin was altered in 2 patients (P3, P5; Table 1). Postsurgery, fasting insulin was elevated in 3 patients (P2, P3, P5), and 1 demonstrated HbA1c ≥6.5% (P5; Table 1). Proinsulin was outside the normal range in 2 patients pre-PIAT and elevated in 3 patients post-PIAT (Table 1), and the preoperative proinsulin-to-insulin ratio and PI/C ratios were elevated in insulin-dependent patients (Table 1). Pretransplant glucose (<90 mg/dL) and normal fasting insulin trended with insulin independence ($P < 0.1$; Table 1), whereas pretransplant FCP, proinsulin, and HbA1c (<5.7%) were not significant, but pretransplant PI/C (<1.43) correlated with insulin independence (*$P < 0.05$; Table 1).

HOMA-IR was altered in 3 of 6 patients before and 4 of 6 after PIAT (Table 1), none of whom were insulin independent posttransplant. Both insulin-independent patients demonstrated normal pretransplant HOMA-β using insulin or C-peptide and similarly using the equation or the calculator; normal HOMA-β using insulin or C-peptide before PIAT was significantly associated with insulin-independent status post-PIAT (Table 1; **Figure S1A** and **B**, **SDC**, http://links.lww.com/TXD/A444).

After transplant, the proposed insulin-independence SUITO threshold value ≥26 was met by both insulin-independent patients but also by 2 of 4 patients who required insulin (P3 and P6; $P > 0.05$; Table 1). Anecdotally, the calculated SUITO values were strikingly similar to the post-PIAT HOMA-β-C-pep equation values (Table 1). Conversely, BETA-2 scores ≥16.2 significantly aligned with insulin independence in our cohort ($P < 0.05$; Table 1), and receiver operating characteristic curve SUITO versus BETA-2 was also significant ($P < 0.05$; **Figure S2**, **SDC**, http://links.lww.com/TXD/A444). Altogether, these estimations suggest that HOMA-β presurgery closely aligns with patient outcomes postoperatively and may be useful for predicting not only insulin independence following transplant but also the functional output of the transplanted cells.

Preserved β-cell Function Correlates With Insulin Secretion and Glucose Excursions Upon Challenge

Functional output was examined in PIAT patients by OGTTs and associated in vivo GSIS. Pretransplant OGTT showed that of CP-PIAT patients tested, all demonstrated glucose responsiveness (Figure 1A, $P = 0.62$ PIAT-Ins versus PIAT + Ins, *t* test; **Figure S3A**, **SDC**, http://links.lww.com/TXD/A444); the associated insulin secretion was low for P6 (Figure 1A; **Figure S3B**, **SDC**, http://links.lww.com/TXD/A444) and more robust in PIAT-Ins patients (Figure 1B, hatched lines). Six months post-PIAT, both insulin-independent patients demonstrated lower glucose excursions during OGTT (Figure 1C) and consequently lower total AUC (**Figure S3C**, **SDC**, http://links.lww.com/TXD/A444) than others tested ($P = 0.051$ PIAT-Ins versus PIAT + Ins, *t* test), whereas P1 demonstrated somewhat overcompensated insulin release (Figure 1D, hatched lines), particularly at 30 min. Similar glucose and AUC trends persisted when reevaluated at 12 mo (Figure 1E, hatched lines; **Figure S3C, E**, **SDC**, http://links.lww.com/TXD/A444; $P < 0.05$ PIAT-Ins versus PIAT + Ins, *t* test); P2 and P4 displayed fasting hyperglycemia (Figure 1E), and P4 released negligible insulin upon OGTT (Figure 1F; **Figure S3F**, **SDC**, http://links.lww.com/TXD/A444). However, limited OGTT data and large variability prevented statistical significance for total secreted insulin AUC between patients post-PIAT ± supplemental insulin (**Figure S3D, F**, **SDC**, http://links.lww.com/TXD/A444).

Patients Who Achieved Posttransplant Insulin Independence Demonstrated Preserved Islet Identity

Within the insulin-independent patient pancreases, the proportion of β-cells dually stained for insulin and glucagon was low (Figure 2A), as were those β-cells that displayed VIM colocalization (Figure 2C). Loss of UCN3 immunostaining was minimal in these 2 biopsies (Figure 2E). Of patients who required insulin post-PIAT, there were diverse variations in identity metrics present. Patient 4 biopsy had a scarcity of endocrine cells available for analysis (**Table 1**, **SDC**, http://links.lww.com/TXD/A444). There were significant elevations in insulin-glucagon colocalization in insulin-dependent patients (Figure 2A, P3, P4, P6), some of whose islets were entirely overlapped in color (Figure 2B, P6), although large variability prevented pooled significance (Figure 2A; $P = 0.24$). There was significantly increased insulin-VIM colocalization in all insulin-dependent patients versus insulin-independent patients (Figure 2C, $P < 0.05$; Figure 2D, VIM, red; insulin, green, arrows) and significantly reduced UCN3 presence in β-cells in insulin-dependent patients (Figure 2E, $P < 0.05$; Figure 2F; Ins, green, UCN3, red, arrow, P6).

Islet morphometry was evaluated to assess the impact of disease state on islet size and distribution. Although observationally there appeared to be reductions in average islet area (**Figure S4A**, **SDC**, http://links.lww.com/TXD/A444), % β-cell area (**Figure S4B**, **SDC**, http://links.lww.com/TXD/A444), and β-cell mass calculations (**Figure S4C**, **SDC**, http://links.lww.com/TXD/A444) in insulin-dependent versus insulin-independent patients, wide distribution within groups prevented statistical significance.

DISCUSSION

Establishing presurgical patient factors that predict transplant success is critically important for surgical paradigms and patient outcomes alike, particularly noninvasive cellular function assessments. In the PIAT patients examined here, normal presurgical HOMA-β values (60%–100%) correlated with insulin independence at 6 mo postsurgery. Our data align with and support other studies showing that pretransplant glycemic variables correlate with postoperative insulin independence after 1 y, particularly fasting glucose[33] and HOMA-β.[31] Here we further interrogate that link using 2 equations to examine graft function and report BETA-2 to be more rigorous than SUITO in correctly predicting insulin independence posttransplant. Importantly, we demonstrate that those patients with poorer pretransplant glycemic variables, including abnormal HOMA-β, demonstrate dynamic loss of β-cell identity histologically and β-cell function by OGTT. Crucially, HOMA-β can be assessed by simple serology noninvasively, which is important because there are currently no modes to directly visualize islet status in vivo, and pancreas biopsy is not routinely, if ever, performed for CP

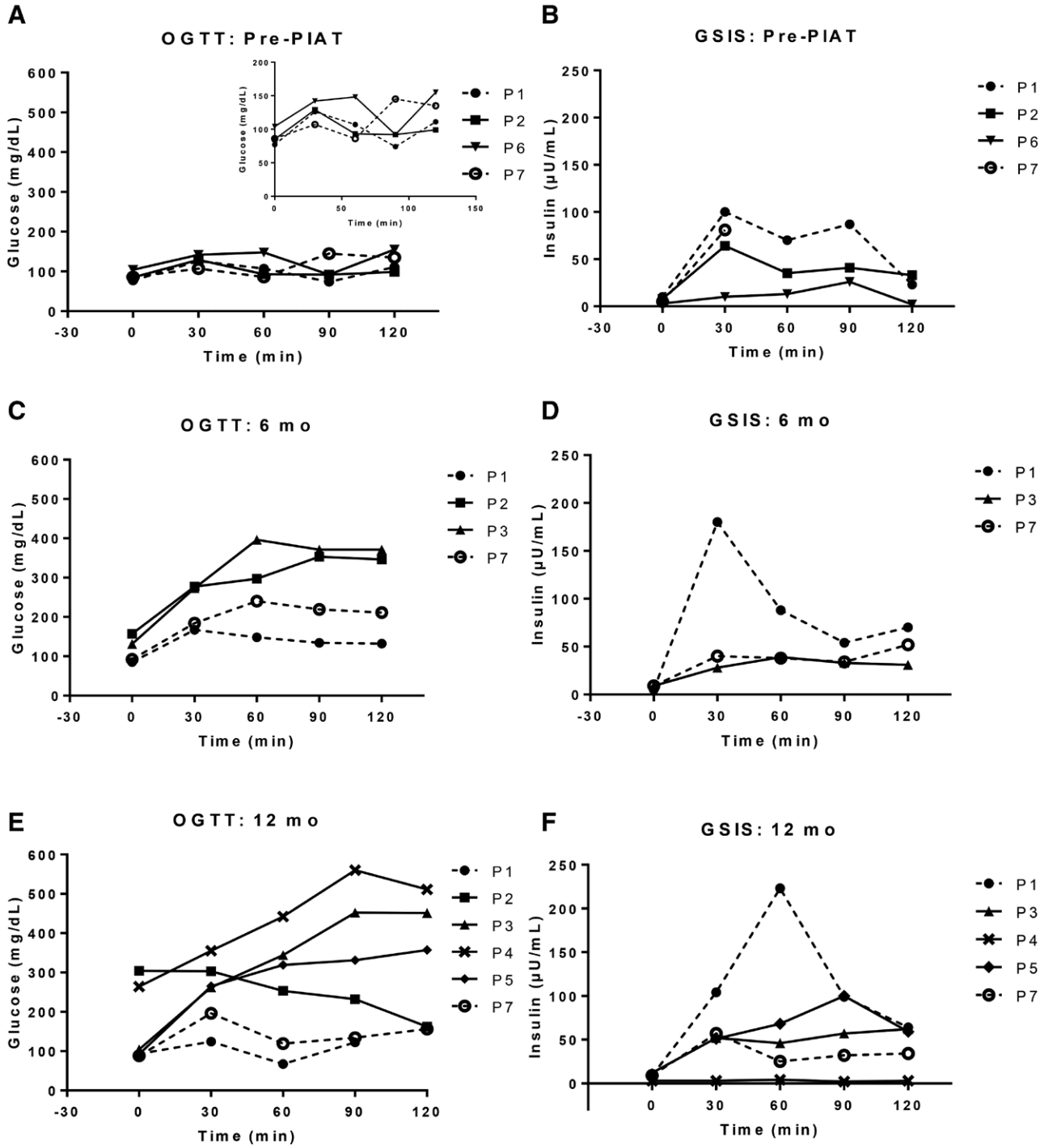

FIGURE 1. OGTTs demonstrate the functional output of islet autotransplantation following PIAT. Comparison of glucose (A, C, E, mg/dL) and secreted insulin (µU/mL) following 75 g ingestion before (A and B), 6 mo (C and D), and 12 mo (E and F) after PIAT. Before transplant, there was no difference in patient glucose (A), but a more robust insulin secretion was noted in those who achieved insulin independence after transplant (B, P1, P7, hatched lines). These same 2 patients self-stratified by lower glucose excursions (C, E, hatched lines) and 1 (P1, closed circle) demonstrated exaggerated insulin release at 6 (D) and 12 (F) mo. GSIS, glucose-stimulated insulin secretion; OGTT, oral glucose tolerance test; PIAT, pancreatectomy and islet autotransplantation.

progression assessment. Noninvasive glycemic testing such as HOMA-β may be combined with other multifactorial preoperative decisions for optimizing postoperative endocrine outcomes by providing predictions or expectations based on patient factors, which supports the reported need to identify optimal patient selection, indications, and timing of PIAT as specified in a National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)-funded workshop.[34]

Insulin injection independence was used here as a binary metric to differentiate success of islet transplants. Sutherland et al[10] reported improvements in health-related quality of life outcomes in all patients post-PIAT but with greater scores in those

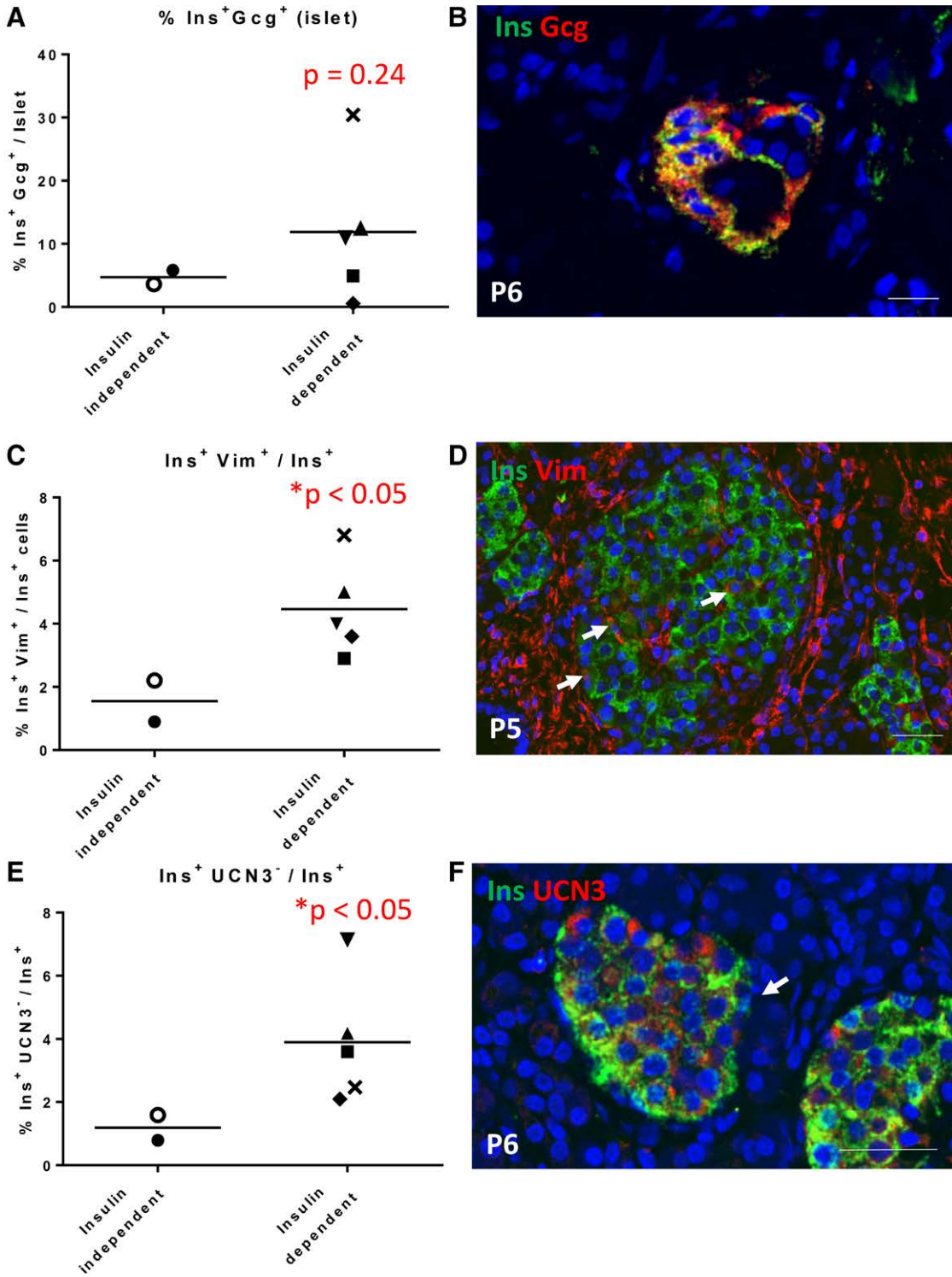

FIGURE 2. Islet identity at the time of PIAT by histological assessment. Islet identity loss was demonstrated in CP patients by dual Ins+Gcg+ cells (A and B), gain of vimentin (C and D, red) in insulin-expressing cells (C and D, green), as well as loss of urocortin 3 (E and F, red, arrow) in β-cells (E and F, green) at the time of PIAT. Line denotes % mean, analyzed by *t* test + Welch correction by insulin-dependence status post-PIAT. Patient symbols are denoted as follows: P1 (closed circle); P2 (closed square); P3 (closed triangle up); P4 (cross); P5 (closed diamond); P6 (closed triangle down); P7 (open circle). Scale bar denotes 50 μm. CP, chronic pancreatitis; Gcg, glucagon; Ins, insulin; PIAT, pancreatectomy and islet autotransplantation; UCN3, urocortin-3; Vim, vimentin.

who became insulin independent postoperatively. C-peptide presence, however, correlates more specifically with islet graft function after 18 mo in T1D patients.[35] This is a nuanced metric of procedural success relative to simple insulin independence, with variable protection from severe hypoglycemia and microvascular complications, afforded by relatively low levels of endogenous insulin production.[19] Only 2 of 7 (28.6%) of our PIAT patients had maintained insulin independence ≥1 y, comparable with other transplant centers.[10,31,33,36] However, the majority of patients examined here (6/7) demonstrated

sustained C-peptide presence following PIAT, indicating overall graft function, even despite glycemic excursions in insulin release at OGTT, and only 1 patient (P4) had daily insulin dose akin to full replacement (~0.55 U/kg[37]). Although it is not unreasonable to assume that the different site of transplantation for P4 ultimately may have influenced function, and optimal transplantation site selection[38] is of critical interest for achieving best outcomes with limited resources, preoperative abnormal/poor glycemic values and HOMA-β estimates in patient 4 infer that the arm versus portal system transplantation site was not the sole or deciding factor of PIAT success. Furthermore, although it is known that higher transplanted islet number largely equates with higher rates of optimal glycemic outcomes posttransplant,[13,14] in our cohort, the IEq/kg numbers were widely disparate between patients and did not translate statistically into this same paradigm. These data support the hypothesis that preoperative islet function, and not (only) transplanted islet number, is a critical factor in PIAT success for postoperative insulin independence. Data from an 8-facility wide, clinical islet transplant consortium phase 3 trial for T1D patients found that quality of the islet products was affected by donor characteristics, and donor pancreas was listed as a critical component of the process.[14]

We have previously documented the differences in islet identity and graft function following PIAT in 3 CP patients who met the ~5000 IEq/kg goal for islet autotransplantation.[12] Moin et al[39] similarly showed loss of hormone presence in islets of patients with CP, as well as endocrine cells that co-expressed insulin and glucagon, and reported β-, α-, and bihormonal cells that expressed the chemokine CCXL10. Our broader analyses here show diverse inter- and intra-CP-patient loss of islet identity; not all patients with diminished graft function exhibit identical histological alterations. Importantly, both postoperative insulin-independent PIAT patients showed a high degree of β-cell lineage maintenance as compared with the 5 insulin-dependent PIAT patients. However, despite some observational changes between groups, we did not identify statistically significant losses in β-cell area, average islet size, or β-cell mass in insulin-dependent patients versus insulin-independent patients. Others have shown that reductions in β-cell area must reach 30%–65%[40,41] for diabetes to occur in CP patients versus nondiabetic controls, which was only evident in 1 patient (P4), and none of our patients were overtly diabetic before PIAT.

Broad consensus supports the link between strong organo-cellular function and better outcomes in a transplant setting. HOMA-β was designed to reflect the responsive interplay between endogenous glucose production and β-cell insulin secretion, specifically at basal (fasting) states in young, healthy individuals.[42] However, CP patients undergoing PIAT necessarily demonstrate variable islet (dys)function as disease progresses. Normal HOMA-β values correlated with insulin independence; we reported these values using both insulin and C-peptide, and using the computer program and the equations, to illustrate the breadth of reported data within the same subject, as well as transparency in data presentation.[43] The ability to identify those patients with well-functioning cells (eg, HOMA-β, 60-0%–100%) and to quantitate these phenomena before surgery by a numerical threshold provides informed patient stratification and could putatively improve surgical modeling estimations for aiding preoperative counseling regarding the timing and decision to proceed to PIAT. Other groups have shown that all patients who met ADA criteria for prediabetes or impaired fasting glucose were insulin- dependent 1-y following PIAT,[31,33] which clearly demonstrates that islet function before and after surgery is paramount for insulin independence. Although all 7 of our patients were considered nondiabetic at the time of PIAT, and few (2/7) met criteria for prediabetes or impaired fasting glucose (P4, P6), only 2 were insulin independent 6 mo post-PIAT. Therefore, these preoperative glycemic cutoffs are likely insufficient to predict optimal outcomes using fasting glucose or HbA1c alone. Interestingly, our findings of normal pretransplant HOMA-β predicting insulin independence was strongly supported by Quartuccio et al[31] but only marginally by Nanno et al[33]; overall, Nanno et al[33] found these metabolic measures correctly predicted insulin independence in ~70% patients. Our histological evidence provides the missing link between serological and surgical findings and allows the detailed integration of information on a patient-by-patient basis. For instance, both P2 and P3 had pretransplant fasting glucose and HbA1c well below the clinical thresholds for diabetes risk, and both patients received ~5000 IE/kg; yet both became insulin-dependent post-PIAT. Histological data show elevated rates of VIM colocalization as well as loss of UCN3 within β-cells in both patients. Importantly, P2 showed high PI/C-peptide ratio, and both P2 and P3 demonstrated elevated HOMA-β values. Patients 5 and 6 showed wider variation in pretransplant glycemic values and low IEq/kg transplanted; these patients were also rendered insulin-dependent postoperatively, and both patients demonstrated poor HOMA-β values pretransplant. These data also support the rationale for evaluating the rigor of the 2 posttransplant graft function equations. Although postsurgical HOMA-β-CPep and the SUITO index scores were anecdotally similar, the reported SUITO threshold value of ≥26 for insulin independence was not robustly supported in our cohort. Conversely, BETA-2 scores ≥16.2 was validated here; others suggest BETA-2 ≥18 to be more reliable for insulin independence.[44]

One confounding variable when determining the purported success of islet transplants may be low-carbohydrate diet (LCD) adherence, with 1 report showing patient BETA-2 scores ≥13 to ≤16 who remained insulin independent by LCD adherence,[20] and others reporting improvements in T1D islet transplantation with LCD plus exercise.[45] In our patient group, 1 insulin-independent patient (P1) post-PIAT had self-imposed LCD; ironically, this may explain the overcompensated GSIS.[46,47] Although curious, this metric is difficult to standardize and is currently only speculative in our small and generally homogenous patient cohort. Indeed, the trends proposed from our small cohort welcome larger analyses in patients of diverse etiology and in a larger context. Although other clinical trials have also found the majority of study participants were similar in age, gender, and ethnicity to ours,[11,31] our patient cohort demonstrates that wide variability exists in islet status irrespective of putative patient similarity. However, this cohort does not represent the population at large and caution should be applied to any broad extrapolation.

Glycemic and surgical endpoints are significantly improved when cellular function as shown by HOMA-β is prioritized, which may have functional utility during clinical patient evaluation.

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