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Context: Islet transplantation (IT) can treat patients with severely unstable type 1 diabetes. Prehepatic kinetics of insulin secretion (ISec) in two phases can be calculated by C-peptide levels during meal tests. We proposed to describe the ISec profile after a mixed-meal tolerance test (MMTT) in IT recipients and to determine whether the calculated ISec indexes can predict graft outcome.

Methods: We analyzed 34 MMTT among 11 patients who underwent IT between 2011 and 2016 and compared them with healthy controls and patients with type 2 diabetes (T2D). ISec indexes and insulin sensitivity were calculated from models of Van Cauter, Breda, and Mari after MMTT. Graft success was defined by total insulin independence without any criteria for diabetes.

Results: In patients with successful IT, the first- and second-phase ISec indexes were lower than those of controls ($P < 0.001$) and did not differ from those of the T2D group. Nevertheless, insulin sensitivity of IT recipients was similar to that of the control group and higher than that of the T2D group. The index of the second phase of ISec ϕS was correlated with total infused islet equivalents (IEQs), was a good predictor of diabetes (re)occurrence, and allowed us to calculate 9500 IEQ/kg as the minimum needed to reach insulin independence.

Conclusion: We showed that indexes from the first and second phases of ISec are altered in insulin-independent IT recipients. Higher sensitivity distinguishes them from patients with T2D. Even in insulin-independent patients, IT remains a marginal mass model. Moreover, ϕS can estimate transplanted islet mass and predict IT recipient outcomes.

Abbreviations: AIR, acute insulin response; BCS, β cell glucose sensitivity; BMI, body mass index; DIR, diabetic islet recipient; GPAIS, glucose potentiation of arginine-induced secretion test; HbA1c, hemoglobin A1c; IEQ, islet equivalent; IQR, interquartile range; ISec, insulin secretion; ISR, insulin secretion rate; IT, islet transplantation; IVGTT, intravenous glucose tolerance test; MMT, mixed-meal test, MMTT, mixed-meal tolerance test; NDIR, nondiabetic islet recipient; OAD, oral antidiabetic drug; T2D, type 2 diabetes.

Islet transplantation (IT) is an interesting and effective clinical strategy to treat patients with severely unstable type 1 diabetes. In contrast to pancreas transplantation, it is a minimally invasive approach to restore a β cell function.

Since the Edmonton protocol (1), the islet mass transplanted has been known to be a strong predictor of insulin independence. However, recent studies do not highlight this correlation (2, 3) and suggest exploring β cell function. Actually, during the process of islet isolation and IT, successive losses of functional β cell mass occur: the isolation procedure with enzymatic destruction harms the islets' support and vascularization, leading to a lack of engraftment (4), the instant blood-mediated inflammatory reaction leads to the destruction of islets $\leq$80% by an inflammatory and coagulation response in the early hepatic transplantation phase (5), and later, graft rejection or immunosuppressive drug toxicity acts directly on β cell functional mass (6). Therefore, monitoring the functional β cell mass after transplantation becomes a priority for IT recipients; it could predict the success and strength of insulin independence. In clinical practice, this evaluation would be helpful in determining the best time to perform an additional graft or to adjust antidiabetic or immunosuppressive treatment.

Islet graft secretion can be challenged by several tests, most performed in physiology departments. The intravenous glucose tolerance test (IVGTT), arginine test, glucose potentiation of arginine-induced secretion test (GPAIS), oral glucose tolerance test, and mixed-meal test (MMT) are the most frequently performed tests. In response to intravenous glucose stimulation, it is well known that the first phase of insulin response is an early marker of β cell dysfunction in type 2 diabetes (T2D) (7) and type 1 diabetes (8). Recent studies (9, 10) have demonstrated that IT can restore to the normal range the first phase of insulin secretion (ISec) [acute insulin response (AIR)] in response to glucose and arginine. β cell secretory capacity derived from GPAIS thus appears to be a strong predictor of the functional β cell mass in the context of autograft and allograft (3, 11) Other studies (12, 13) showed an impaired first-phase insulin response to MMTs in these patients. In both islet autografts and allografts, AIR to intravenous arginine is well correlated to islet mass, whereas AIR to intravenous glucose is the best predictor of glycemic control in allograft recipients (3, 14, 15).

Current model-derived calculations of ISec based on C-peptide kinetics during a meal test allow us to measure the two phases of ISec and insulin sensitivity (16–19). In these conditions, the models of Breda *et al.* (17) and Mari *et al.* (19) are well accepted and reproducible. The second phase, expressed as β cell glucose sensitivity (BCS), has been shown to be a strong predictor of diabetes progression (20), and recent studies also suggest that it is closely related to the pancreatic β cell mass (21) in a partial pancreatectomy model. However, the description of the two phases of ISec according to these models has not been performed in the IT context.

We therefore suggest using this mathematical model analysis to study IT recipients. We chose to perform a mixed-meal tolerance test (MMTT) using our model of standardized breakfast test (22) to more physiologically stimulate the β cells. The aim of this study was to describe IT recipient ISec and to compare their data with those of nondiabetic controls and patients with T2D available in our department. We also wanted to assess whether the results of these tests could predict transplanted islet mass and clinical outcome in these patients.

Patients and Methods

Patients and transplantations

Islet-allografted patients were recruited from the cohort of the Groupe de Recherche Rhin Rhĵne Alpes Genève pour la Transplantation d'Ilots de Langerhans 1c and 2 (n = 4) and Trial Comparing Metabolic Efficiency of Islet Graft to Intensive Insulin Therapy for Type 1 Diabetes's Treatment (n = 7) clinical trials (23, 24). They underwent IT in the Hospital of Montpellier under standard inclusion and exclusion criteria according to previously reported procedures (25) and immunosuppression as recommended by each clinical trial. Eight patients underwent IT alone, and three underwent IT after kidney transplantation. Each transplant protocol allowed patients follow up through regular metabolic testing; our center chose MMTT as the stimulation test. Islet mass was estimated by the sum of known islet equivalents (IEQs) per kilogram received during each infusion, and our analysis was limited to the tests carried out within 2 years of the first transplant for reliable estimation of IEQ (the hepatic survival of islets over time remains unclear). Results of MMTT from our database were used to compare IT recipients. We thus present four different metabolic profiles for analysis: healthy nondiabetic control subjects, patients with T2D, and IT recipients with full success [nondiabetic islet recipients (NDIRs)] or still diabetic [diabetic islet recipients (DIRs)].

Success definition

Islet graft full success was defined according to the following criteria: insulin independence, no oral antidiabetic drug (OAD), hemoglobin A1c (HbA1c) $\leq$6.5%, positive C-peptide (>0.5 ng/L), and plasma glucose level <7 mmol/L under fasting conditions and <11.1 mmol/L 2 hours after MMTT. In our analysis, islet-grafted patients who met all these criteria were included in the NDIR group. The others were in the DIR group.

MMTTs

All tests were performed at Montpellier University Hospital. Subjects fasted overnight before being tested. Insulin-dependent

subjects suspended long-acting insulin for 24 hours and short-acting insulin for 12 hours before being tested. They had a standardized breakfast test meal within 15 minutes that contained 465 kcal including 76 g of carbohydrates (63.4% carbohydrates, 27.5% lipids, and 9.1% proteins with bread, butter, sugar, and coffee). Blood samples were collected at T0 and then at 15, 30, 60, 90, 120, 150, 180, and 210 minutes after ingestion to measure plasma glucose, C-peptide, and insulin.

Mathematical analysis

Calculation of ISec

The C-peptide kinetic response to the MMTT enables determination of the insulin secretion rate (ISR), as described by the two-compartment model originally proposed by Eaton *et al.* (26) and further improved by Van Cauter *et al.* (27), in which the model parameters were individually adjusted to the subject's anthropometric data. This β cell response was then quantified by two most widely accepted models available in the literature: Breda's and Mari's (17, 19). We analyzed the first phase of ISec by using the rate sensitivity k1 (pmol/m^2/mmol), according to Mari, which is the dynamic dependence of ISec on the rate of change of the glucose concentration. The second phase of ISec was estimated by BCS, described by Mari and calculated as the slope (pmol/min/m^2) of the relationship between ISR and glucose concentration, and by the ϕS index, described by Breda, including the insulin second-phase response to the meal test challenge. ϕS is defined as the basal average static phase ISec per unit over basal average glucose concentration (18).

Insulin sensitivity

Insulin sensitivity was calculated with the oral minimal model described by Caumo *et al.* (16) and validated by our team (22). This widely validated procedure extends to standard meal and oral glucose tolerance tests the classic Bergman minimal model initially developed for IVGTT (28) and based on the analysis of changes in plasma glucose and insulin concentration after glucose challenge. A disposition index was also calculated, by analogy with Bergman *et al.* (28), by multiplying total ISec by insulin sensitivity.

Statistical analysis

Patient characteristics (n = 11) and islet graft results were expressed as the median and interquartile range (IQR) (Q1; Q3). Data from meal tests were expressed as the mean ± standard deviation. Data from two groups (NDIR and DIR) were analyzed by unpaired *t* tests, and multiple groups (controls, T2D, NDIR, and DIR) were compared with the Kruskal-Wallis test. Because the success or failure of islet graft was evaluated several times for each patient, we modelized the association between each score and the success as defined according to a generalized linear mixed-effects model for repeated measures. The threshold of significance was set at 5%.

Results

Subject characteristics

Before islet graft, the median age of the 11 patients was 49 years (IQR 43.5; 56), with a median HbA1c of 8.1% (IQR 7.95; 8.65) while taking 0.45 (IQR 0.33; 0.54) U/kg insulin per day. Between 2001 and 2016 they underwent 2 (n = 6) or 3 (n = 5) islet infusions, corresponding to 14,070 ± 3084 IEQ/kg body weight infused. Recipient characteristics at baseline, IT data, and patient data at 6, 12, and 24 months after transplantation are available in the Supplemental Data. All 10 subjects studied at 12 months had an HbA1c ≤7% (median: 6%, IQR 5.6; 6.2) with a positive C-peptide. Two recipients still needed insulin therapy (one acute rejection and one incomplete graft) even though they experienced reduced insulin requirements, improvement in HbA1c, and elimination of severe hypoglycemic events. During follow-up, 20% resumed insulin therapy or OADs 2 years postgraft. Before islet graft, the glomerular filtration rate was higher in patients undergoing IT alone than in patients undergoing IT after kidney transplantation (median: 97.5 and 66 mL/min, respectively). Creatinine clearance decreased at the beginning of immunosuppressive treatments in the IT alone group and then remained stable during the 2 years of follow-up. During the MMTT, immunosuppression consisted of tacrolimus with a target trough levels of 6 to 10 ng/mL for all patients except one, associated with either mycophenolate mofetil (3 DIR and 5 NDIR) or sirolimus (1 DIR and 2 NDIR). No patients underwent steroid therapy except one in the NDIR.

Demographic data for IT recipients with success (NDIR) or failure (DIR) of the graft and comparators [healthy nondiabetic controls (n = 127), patients with T2D (n = 47)] were comparable in sex and age (Table 1). Thirty-four MMTTs were performed in the 11 islet-grafted patients; 19 were analyzed as conditions previously defined as successful. The control group had significantly lower fasting plasma glucose than the DIR, NDIR, and T2D groups ($P < 0.0001$). No difference in body mass index (BMI) was observed between healthy controls and islet-grafted patients. In islet-grafted patients, the NDIR group was significantly younger than the DIR group during MMTT ($P = 0.0004$) and received a significantly larger quantity of islet (IEQ/kg, $P = 0.042$) (Table 1).

Plasma profiles after MMTT

The glucose, crude insulin, C-peptide, and calculated ISR profiles are shown in Fig. 1. As previously described for successful transplants, the plasma glucose profile of the NDIR group remained <11.1 mmol/L after MMTT but significantly higher compared with controls: blood glucose increased to 10.0 ± 2.3 mmol/L *vs* 6.2 ± 1.6 mmol/L at 90 minutes in healthy controls ($P < 0.001$). Blood glucose profiles showed obvious diabetes in the case of T2D and DIR, with a fasting value of 8.5 ± 2.9 mmol/L and 8.8 ± 3.8 mmol/L, increasing to 14.6 ± 3.5 mmol/L (T90) and 18.0 ± 8.2 mmol/L (T210), respectively. Crude C-peptide profiles showed significantly

Table 1. MMTs: Characteristics of Patients and Comparators

	NDIRs	DIRs	T2D	Controls
MMTT, n (male/female)	19 (1/18)	15 (11/4)	47 (22/25)	127 (30/97)
Patients				
Age, y	45.9 ± 6.0	55.6 ± 8.3	56.7 ± 15.1	45.3 ± 14.6
Weight, kg	66.8 ± 9.4	64.1 ± 6.3	91.6 ± 29.2	68.1 ± 11.5
BMI, kg/m^2	24.6 ± 3.5	23.0 ± 2.1	32.8 ± 9.2	24.8 ± 3.2
Fasting blood glucose, mmol/L	5.4 ± 0.4[a]	8.8 ± 3.8	8.5 ± 2.9	4.8 ± 0.5
HbA1c, %	5.9 ± 0.3[b]	7.2 ± 0.9		
Islet grafts				
Infusion number/MMTT	2.37	2.13		
Insulin before graft, U/kg/d	0.51 ± 0.13	0.45 ± 0.14		
Delay after 1st graft, d	663 ± 94	802 ± 691		
IEQ/kg transplant	14,027 ± 1764[a]	11,767 ± 4052		

Data are expressed as mean ± standard deviation. Results of MMTT were compared between IT recipients with (DIR) or without (NDIR) diabetes and patients with T2D and healthy subjects (controls).

[a] $P < 0.05$.

[b] $P < 0.001$ for statistical analysis of DIR *vs* NDIR.

lower values in the NDIR group compared with the control group at every time after MMTT. Despite insulin independence, the peak insulin and C-peptide responses were significantly delayed in the NDIR group compared with healthy controls, as was also observed in patients with T2D. In the DIR group, biological assays of insulin and C-peptide were even lower than C-peptide levels in the T2D group but were nonzero and responded partially and variably to MMTT stimulation (C-peptide at T0 = 1.2 ± 1.4 ng/L and peak at T210 = 2.9 ± 3.0 ng/L).

ISec and insulin sensitivity analysis

The ISR profile, calculated from the kinetics of C-peptide, is shown in Fig. 1D. In healthy control subjects, we confirmed two ISec phases, with a first part up to 60 minutes after MMTT and a second part from T60 to T120. In both the T2D and NDIR groups, ISR increased progressively, and the maximum value appeared at T120. A clear loss in the first phase of ISR was observed in these two groups. It was quantified by the k1 index, according to Mari's model, which was lower in the NDIR (179 ± 38 pMol/m^2/mmol) and T2D groups (209 ± 35 pMol/m^2/mmol) compared with the control group (1025 ± 96 pMol/m^2/mmol, $P < 0.001$). First-phase ISec islet-grafted patients without diabetes was about 17.5% of that in the control group.

A significant decrease in second-phase ISec indexes was also seen in IT recipients. It was quantified by ϕS (Breda's model), which separated controls from the NDIR group, showing lower values ($98.5 \pm 64.9 \times 10^{-9}$ *vs* $26.4 \pm 18.8 \times 10^{-9}$ respectively, $P < 0.001$) and the DIR group ($4.1 \pm 3.7 \times 10^{-9}$, $P < 0.001$) (Fig. 2A). BCS, another way to quantify the second phase of ISec, was logically also lower in the NDIR group (63.3 ± 84.6 pMol/min/m^2) compared with controls (177.8 ± 90.7 pMol/min/m^2, $P < 0.001$) and much lower in the DIR group (13.4 ± 12.4 pMol/min/m^2) than in the NDIR group ($P < 0.001$, Fig. 2B). Indeed, for the NDIR group, second-phase of ISec was only 26.8% of the ϕS and 35.8% of the BCS of healthy controls. This decrease was comparable to the T2D group, in which second-phase of ISec was 26.9% and 36.1% of that of controls, according to the model chosen (Mari or Breda). Insulin sensitivity was no different between the NDIR, DIR, and control groups even if DIR patients tended have higher values. In T2D patients, insulin sensitivity was significantly lower than in the control and NDIR groups ($4.4 \pm 5.8 \times 10^{-4}$ μU/mL per minute *vs* $11.1 \pm 8 \times 10^{-4}$ and $9.3 \pm 9.3 \times 10^{-4}$ μU/mL per minute, $P < 0.001$) (Fig. 2C). This decrease was also found with the disposition index in patients with T2D (93 ± 160) as compared with the control (1163.6 ± 1372, $P < 0.01$) and NDIR groups (224.2 ± 211, $P < 0.01$) (Fig. 2D).

Correlation of index from the second phase of ISR to islet-grafted mass and function

We analyzed the relationship between islet-grafted mass and the markers of the second phase of ISec (ϕS and BCS) in 22 MMTTs within 2 years of the first transplant. These relationships are shown in Fig. 3. Both ϕS and BCS are strongly and significantly correlated to IEQ/kg infused, with a higher correlation coefficient for ϕS ($r = 0.78$, $P < 0.0001$) than BCS ($r = 0.705$, $P = 0.0002$). The scatterplots of ϕS for patient follow-up after first transplant are shown in Fig. 4. We confirmed the relationship to islet mass by highlighting an increase in ϕS after an additional infusion of islets. We noted a gradual decrease in ϕS from the last transplant to the end of follow-up except in one patient, in whom ϕS increased. This finding could be explained by weight loss observed at the same time in this patient. As we previously showed, ϕS was significantly higher in the NDIR

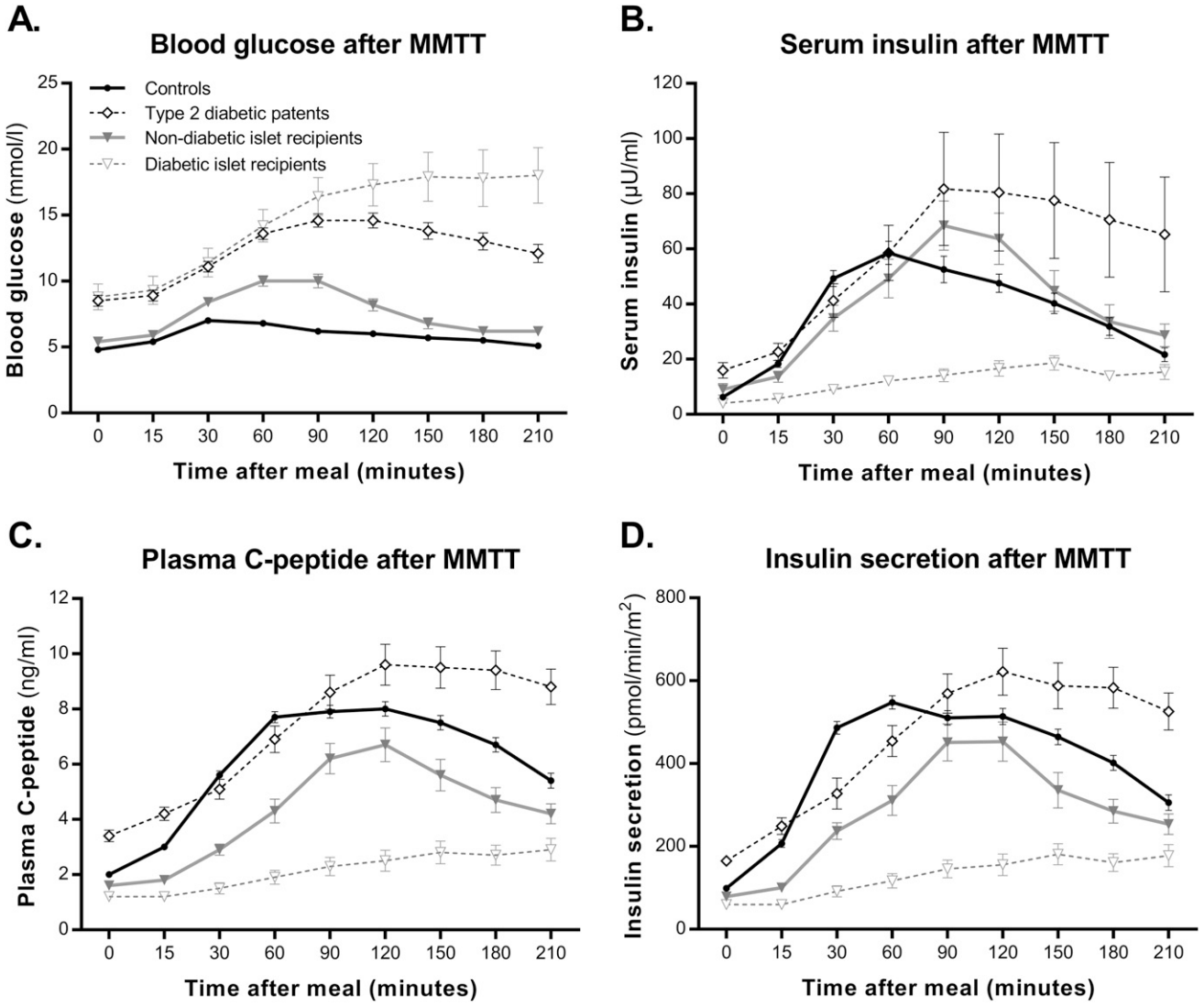

Figure 1. Plasma profiles of (A) glucose, (B) crude insulin, (C) C-peptide, and (D) ISec rate calculated from the C-peptide kinetics in responses to MMTTs redesigned as described by the two-compartment model of Van Cauter *et al*. (27) are shown for the control, T2D, NDIR, and DIR groups. Results are expressed as mean ± standard error.

group (range 12.7 to 42.1 $\times$ 10^{-9}) than in the DIR group (range 0.04 to 10.37 $\times$ 10^{-9}; $P < 0.001$). In fact, ϕS enabled us to perfectly distinguish between success and failure of the islet graft: below a cutoff value of 10.37 $\times$ 10^{-9}, patients resumed diabetes with a sensitivity and specificity of 100%. An additional analysis was performed by separating patients using OADs or insulin therapy (Fig. 4B) and showed no difference between these two subgroups. Given the strong correlation between ϕS and infused islet mass, the value of ϕS at 10.37 $\times$ 10^{-9} corresponded to 9500 IEQ/kg infused, which we calculated as the minimum infused islet dose needed to reach insulin independence. According to a generalized linear mixed-effects model for repeated measures, the threshold ϕS value of 10.37 was significantly associated with insulin independence (odds ratio = 1.11; 95% confidence interval, 1.03 to 1.19; $P = 0.0045$).

Discussion

This study provides evidence that although it provides an almost complete reversal of type 1 diabetes, IT results into altered levels of ISec, with a clear decrease in the two phases of ISec that are markedly lower than those of healthy controls.

MMTs used in our study elicited responses in ISR that were similar to those found in previous reports in IT with other stimulation tests. Our study used mathematical models, such as those of Mari and Breda, to analyze and quantify ISec in an IT context. We confirmed a single-phase ISec after MMTT in IT recipients with the absence of the first phase (k1 index in our study) as it was previously reported by Rickels *et al.* (13), with K_G (intravenous glucose disposal rate) calculated after IVGTT, incremental C-peptide response after MMT or arginine test, and glucose-potentiation slope after GPAIS. Hirsch

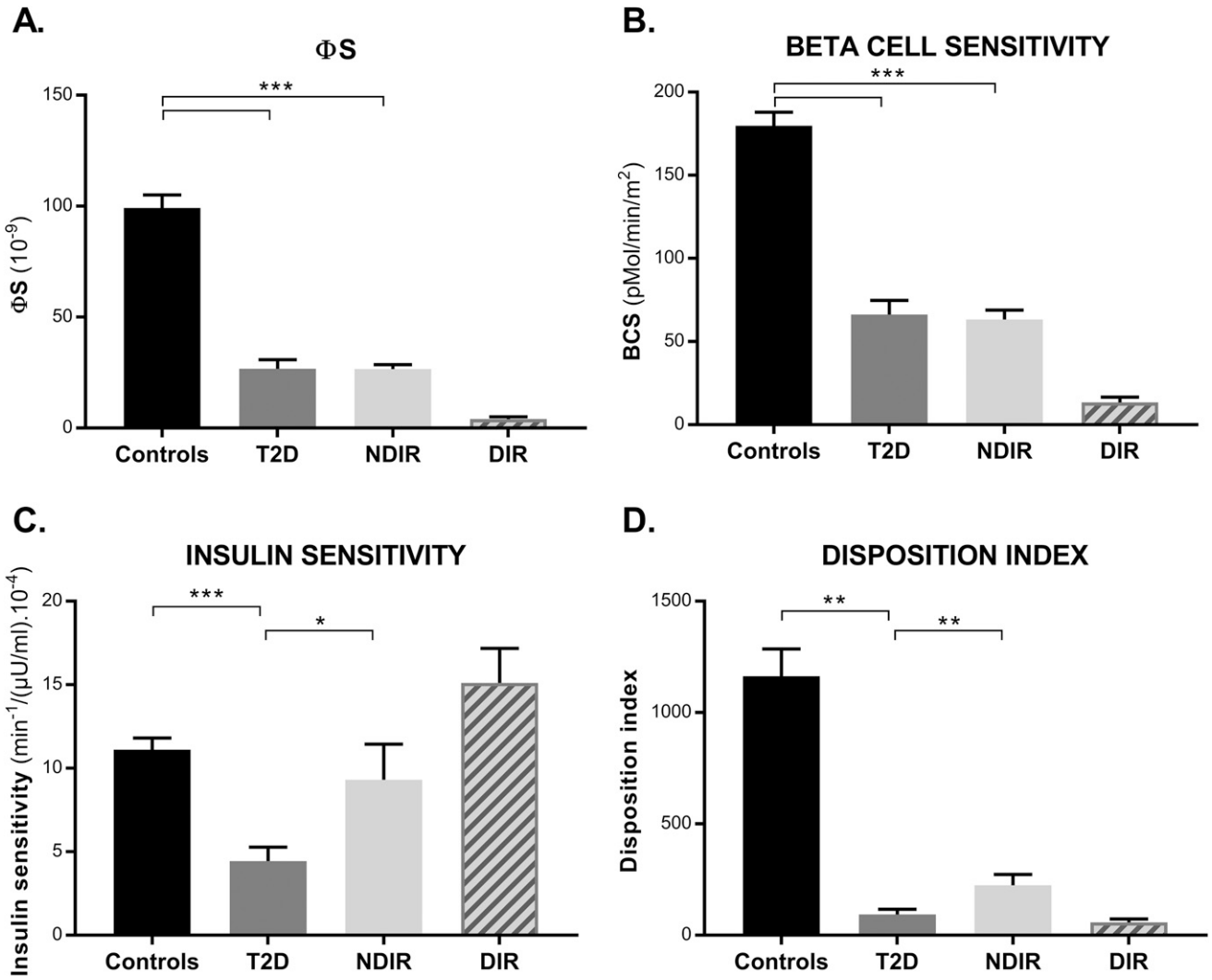

Figure 2. Parameters of the second phase of ISec and insulin sensitivity calculated after MMTT. In each group, the second-phase ISec indexes were calculated after MMTTs from two distinct models: (A) Breda's as ϕS and (B) Mari's as BCS. (C) Insulin sensitivity and (D) disposition index were quantified according to Caumo's model in control healthy subjects (controls), patients with T2D, NDIRs, and DIRs. Bars represent means with standard errors. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

et al. (3) showed similar results on the AIR in response to IVGTT, arginine test, or GPAIS. Rickels highlighted a normal first-phase response to intravenous glucose and arginine in IT (9, 10) when a sufficiently robust islet mass survived after engraftment. Nevertheless, he emphasized that β cell functional defects seen after IT were explained by a low functional β cell mass. We reported here the same observation. Indeed, we showed a markedly lower (60% to 80%) second phase of ISR (BCS and ϕS) in NDIRs compared with controls. These data are also in agreement with a reduction of 67% in the area under the curve of insulin in response to IVGTT observed by Ryan *et al.* (29) in islet-grafted patients *vs* controls, despite the infusion of ≥850,000 IEQs (*i.e.*, 85% of estimated controls' mass). In islet autotransplantation, Teuscher *et al.* (11) and more recently Robertson *et al.* (15) also highlighted a 60% to 71% reduction in AIR peak value response to GPAIS.

In NDIR, the first- and second-phase ISec was decreased, as can be observed in other metabolic dysregulation cases such as patients with T2D. The comparison with data from patients with T2D is also a feature of our study.

Finally, our data indicate impairment in ISec in all islet-grafted patients, those with total insulin independence, and a greater impairment in those with diabetes. In IT, the range of values of model-derived ISec indexes appears to be within the range of marginal mass. However, such a pattern of ISec can lead to effective insulin independence in islet-grafted patients because their insulin sensitivity is normal, whereas in patients with T2D who are insulin resistant it cannot result in normoglycemia. Despite infusion of >10,000 IEQ/kg per patient, which theoretically represents 60% of the islet mass in healthy subjects, only 20% to 30% of insulin function is seen. Instant blood-mediated

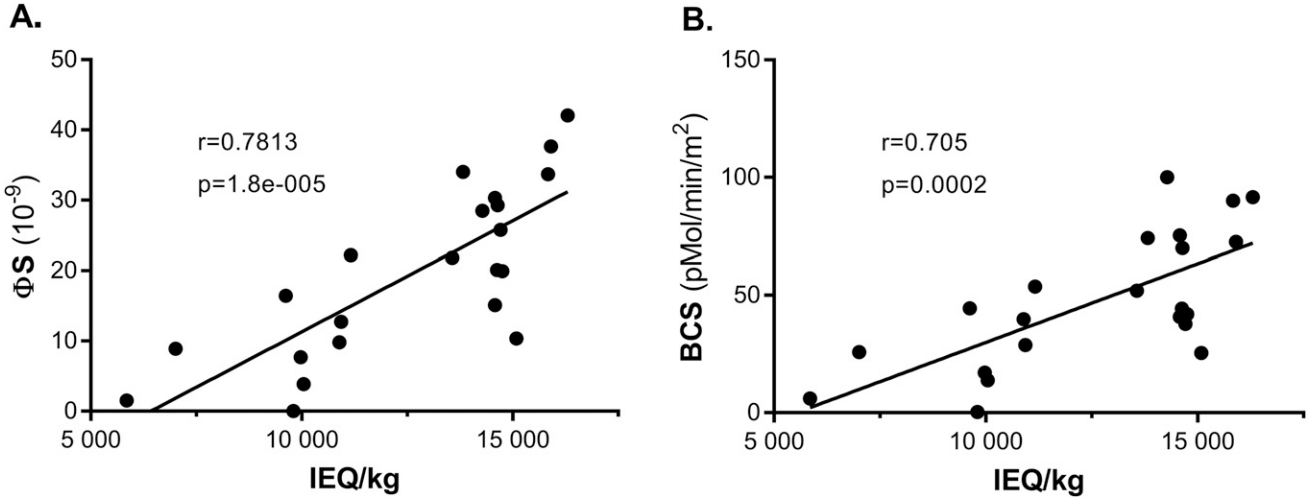

Figure 3. Relationship between islet mass and parameters from second-phase ISec. A relationship between islet mass (IEQ/kg) and parameters from second-phase ISec (A) ϕS and (B) BCS calculated after MMTT was observed in islet-grafted patients within 2 years of their first infusion.

inflammatory reaction, hypoxia, and initial glucotoxicity may explain this 50% insulin function loss. These data support the previous report by Rickels *et al.* (9, 30) showing that impaired β cell secretory capacity in IT was explained more by a low engrafted β cell mass than by a deleterious effect of immunosuppressive drugs. IT improved insulin sensitivity, and glucocorticoid-free immunosuppression with low-dose tacrolimus or sirolimus does not induce insulin resistance (9).

Currently, many stimulation tests are available to explore β cell function. Recently, MMTTs showed a good reproducibility in different metabolic profiles. The ϕ total is significantly associated with AIR to IVGTT, and insulin sensitivity from MMTTs or IVTTs is well correlated (22). In conditions of low islet β cell mass, MMTT induces a higher C-peptide response compared with IVGTT (31) or glucagon tests (32), with better clinical tolerance. Indeed, breakfast tests induce a physiologic ISec including both stimulation by carbohydrate, fat, and protein with macronutrients and an incretin effect. Moreover, mathematical models as proposed by Mari and Breda enable a finer analysis of ISec by including peripheral insulin sensitivity, specific to each patient, and results from these models can change with time in the same individual, as evidenced by weight loss. These models are well validated and used in clinical research, but to our knowledge they had never been used in the islet graft context. We used C-peptide kinetics to quantify ISec. It seems to be a better approach than plasma insulin assay because in islet graft, β cells are located in the liver, and insulin extraction remains unknown in this context. Although there were no

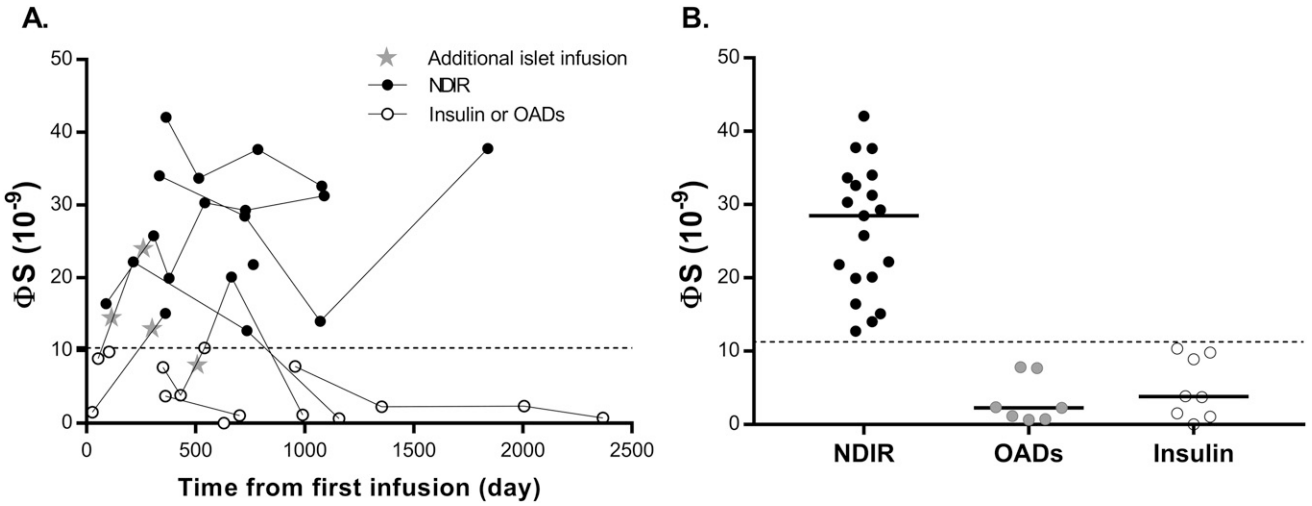

Figure 4. (A) Temporal evolution of second-phase ISec according to ϕS. ϕS calculated in 11 patients after their first IT showed a clear distinction between islet-grafted patients according to metabolic status. Stars show additional islet infusions. (B) All patients without diabetes had a ϕS $>10.37 \times 10^{-9}$, whereas all patients who resumed OADs (n = 7) or insulin (n = 8) had a ϕS under this threshold. The horizontal dotted line represents this cutoff value. Solid horizontal lines in each group represent the medians.

statistical differences in the parameters of MMTTs calculated from C-peptide kinetics between insulin-independent islet graft recipients with or without renal failure (data not shown), it is important to note that reduced renal blood flow increases the half-life of C-peptide and could overestimate ISec (33). Furthermore, a kidney-only or other solid organ recipient group could be studied in the future to determine the implications of kidney dysfunction and immunosuppressive therapy for C-peptide and glucose metabolic status.

In this study, we demonstrated that second-phase indexes of ISR (ϕS and BCS) are more specific and sensitive in unmasking differences in functional β cell mass between controls, islet recipients remaining insulin independent, and those resuming insulin. In fact, recent literature has already highlighted its pathophysiological relevance because it is a major determinant of glucose tolerance (34), and it can predict and track the evolution to diabetes (20). In our analysis, a ϕS index $>10.37 \times 10^{-9}$ demonstrated perfect discrimination between those who are insulin independent and those needing insulin or an OAD. We defined the success of IT by both absence of diabetes (fasting blood glucose <7 mmol/L or 120 minutes after MMTT <11 mmol/L) and insulin independence with HbA1c <6.5%, which seems more robust than insulin independence alone. None of the other model-derived indexes used in this study, such as BCS, disposition index, ISR peak value, and total k1 (data not shown), is able to better discriminate these two groups. AIRs to IVGTT and GPAIS were reported to be the most accurate methods to determine suboptimal islet allograft, but their sensitivity (88.2% and 100%, respectively) and specificity (85.9% and 83.3%, respectively) to identify patients needing insulin or not (3) are lower than those of ϕS, which in our study showed a sensitivity and specificity of 100%. To date, in IT, no method has been validated as the gold standard to test our threshold value of 10.37×10^{-9}. The β cell secretory capacity derived from GPAIS was validated to assess the functional β cell mass. Therefore, it should be interesting to explore this question in a larger cohort and compare our MMTT with GPAIS performed in the same cohort. In fact, because this index ϕS declines in parallel with islet function and increases after each islet infusion, it could be valuable in clinical practice to monitor islet-grafted patients to determine the need for additional islet infusions or to compare strategies to prevent islet loss.

β cell mass remains difficult to quantify in clinical practice. Meier *et al.* (21) reported an alteration in second phase of ISec (using BCS according to Mari's model) proportional to pancreatic β cell mass, which was quantified on pancreatic surgical biopsies. In our study, we also found a strong correlation between this index BCS, -quantifying the second phase of ISec, and islet mass (IEQ/kg infused), but this correlation was even higher with the ϕS of Breda's model. To our knowledge, few studies have found a relationship between scores or stimulation tests and β cell mass. After islet autotransplantation, AIR peak value calculated after GPAIS is well correlated to the amount (IEQ/kg) infused (range = 0.81 to 0.91, $P < 0.001$) (11, 15), but it is less significant in islet allotransplantation (r = 0.789, P = 0.011) (14). C-peptide response, 90 minutes after MMTT, also shows a correlation to alloinfused islet mass (r = 0.643, $P < 0.001$) (12), which was confirmed in our study, but this relation is lower than ϕS (data not shown). Finally, using the cutoff value of 10.37×10^{-9} for the ϕS, we were able to calculate that <9500 IEQ/kg grafted, insulin independence is unlikely. This estimate is consistent with the very first Edmonton protocol (1), which suggests a minimum mass of 10,000 IEQ/kg to optimize islet graft results, and with the 9000 IEQ/kg proposed later by Ryan *et al.* (29). However, it should be noted that graft function also depends on islet isolation (purity, cold ischemia), donors (age, BMI, comorbidity), and the metabolic profile of the recipient (BMI, treatment). All these uncontrollable parameters can explain the poor relationship between IEQ/kg and insulin-independent islet graft recipients. It emphasizes the importance of having a simple and validated test available to estimate the functional quality and quantity immediately after the graft and later in the follow-up of IT recipients. Meal tests seem to check these criteria and could be proposed routinely in the follow-up of our IT recipients.

In conclusion, although islet graft success is real from a clinical point of view, we highlight a severe impairment of islet function in response to MMTTs, with alterations in the first and second phases of ISec. The ϕS index perfectly distinguishes insulin-independent islet graft recipients from other patients and is strongly correlated with islet mass. In the absence of a gold standard, we therefore propose a threshold index of ϕS 10.37×10^{-9} to define the critical limit under which glycemic disorders reappear. These results suggest that ≥9500 IEQ/kg is needed to hope for complete IT success. Our approach using ϕS calculations from MMTTs indicates that increasing transplanted and surviving islet β cell mass should be priorities for improving the functional outcomes of islet grafts. Future research should compare ϕS with other tests for predicting functional islet mass

maintenance at precise follow-up times or after incretin treatment initiation.

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