Comparison of Metabolic Responses To The Mixed Meal Tolerance Test Versus The Oral Glucose Tolerance Test After Successful Clinical Islet Transplantation

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Some of this data was presented at the ADA meeting 2014: Koh AI, Dinyari P, Malcolm A, Shapiro AMJ, Senior PA. Mixed Meal Tolerance Test (MMTT) vs. Oral Glucose Tolerance Test (OGTT) after Successful Clinical Islet Transplantation (CIT). Diabetes. 2014;58 (Supp 1): 1955.

Following islet transplantation, mixed meal tolerance tests (MMTs) are routinely utilised to assess graft function, but how the 90-minute MMTT glucose value relates to a 120 minute glucose concentration of ≥11.1mmol/L used to diagnose diabetes following a standardised 75g-OGTT, is not known. We examined this relationship further.

Thirteen subjects with Type 1 diabetes and stable transplant grafts, not on exogenous insulin with HbA1c<7% (53mmol/mol), were studied on 17 occasions with paired OGTTs and MMTTs. Receiver operating characteristic (ROC) curves were constructed to derive the 90-minute MMTT glucose threshold associated with a 120-

minute glucose concentration following a 75g-OGTT ($OGTT_{120}$) ≥11.1 mmol/L and their diagnostic accuracy. Studies with $OGTT_{120}$ ≥11.1 mmol/L (n=5) had diminished C-peptide: glucose, greater integrated glucose and diminished insulin: glucose area under the curve (AUC) ratios (0-120 minutes) and disposition indices; all $p<0.05$, contrasting with MMTTs where no difference in the 90-minute glucose concentrations, C-peptide:glucose, integrated glucose, C-peptide and C-peptide: glucose AUCs (0-90 mins) was seen; all $p>0.05$. A 90-minute MMTT glucose concentration ≥8.0 mmol/L demonstrated a sensitivity and specificity of ≥80% for the diagnosis of $OGTT_{120}$ ≥11.1 mmol/L; area under ROC curve (mean±SEM) 73±13%. A 90-minute MMTT glucose ≥8.0 mmol/L, identifies islet transplant recipients who may require closer monitoring for graft dysfunction.

Abbreviations

Area under Receiver Operating Characteristic - AUROC; Disposition Index – DI; Islet after Kidney – IAK; IFG – impaired fasting glucose; IGT – impaired glucose tolerance; Islet Equivalent Units – IE; Islet Transplantation- ITx; Mycophenolate Mofetil - MMF; Mixed Meal Tolerance Test - MMTT; Normal Glucose Tolerance – NGT; Receiver Operating Characteristic – ROC; UK Islet Transplant Consortium - UK ITC.

Introduction

Islet transplantation (ITx) is an effective treatment for patients with Type 1 diabetes with frequent, severe hypoglycaemia associated with impaired awareness of hypoglycaemia even in the absence of completely normal glucose tolerance or

insulin independence (1-5). Intrahepatic ITx can achieve short-term insulin independence in almost all cases. Factors promoting ITx success include islet number (1, 6) and purity, (7) but long term insulin independence is difficult to maintain (2, 8, 9). Attrition in graft function is well recognised although poorly understood. Mechanisms for the diminished graft function observed with time may include autoimmune and alloimmune processes and an intrahepatic environment that is toxic as a consequence of exposure of islets to high concentrations of immunosuppressive drugs; the relatively low oxygen concentration in the liver may also play a role (10). Monitoring of graft function (11) is therefore of importance in the follow up care of patients to identify individuals who may require metabolic or immunologic support to prevent further graft loss.

Measures of beta cell function may be made directly from circulating C-peptide concentrations (12) after stimulation with arginine (13) and glucagon tests (14) or indirectly using surrogate measures, for example using continuous glucose monitoring systems (15, 16). Recently it has been demonstrated that a measure of ITx engraftment may be derived from a fasting C-peptide measurement (11) but islet transplantation programmes across the world have long recognised stimulated C-peptide measurements as an appropriate primary outcome measure (2, 4, 17, 18). The most used method for stimulating C-peptide response in islet transplant recipients in clinical settings is the mixed meal tolerance test (MMTT) as it is highly reproducible and represents a robust but physiological stimulus for C-peptide secretion with a lower risk for hyperglycaemia, because a smaller load of glucose is used, as compared to an OGTT (19). In the MMTT, a liquid meal is ingested in the fasting state with timed measurements of C-peptide and other metabolites post-prandially. In islet transplantation programmes, the post-prandial C-peptide

concentration at 90-minutes is taken to reflect the peak stimulated circulating C-peptide concentration and is interpreted in the context of the glucose concentration at this time point (20).

The purpose of metabolic testing with MMTT after islet transplantation is to assess graft function rather than to define glucose intolerance or recurrence of diabetes. This is in contrast to the use of OGTT to diagnose degrees of glucose intolerance including impaired fasting glucose, impaired glucose tolerance and post-transplant diabetes in other transplant settings (21), using the same thresholds defined by the WHO (plasma glucose ≥11.1 mmol/L (200mg/dL) 2hours after a 75g oral glucose load) to diagnose diabetes in the general population (22) (See Supplemental Methods – WHO Classification of Glucose Intolerance). The presence of diabetes nevertheless indicates inadequate insulin secretion (in absolute or relative terms) to maintain euglycaemia (23-26). Thus the excursions in glucose and C-peptide after either a MMTT and OGTT reflect beta cell capacity.

Since OGTTs are not performed routinely and no previous studies have explored the responses to both MMTT and OGTT in islet transplant recipients we sought to compare the metabolic responses to MMTT and OGTT in a small cohort of insulin-independent islet transplant recipients who had undergone both tests. This also afforded the opportunity to determine the glucose thresholds at 90-minutes following a MMTT that corresponded to the threshold for diagnosis of diabetes after a 75g-OGTT.

Research Design and Methods

In the original Edmonton Protocol, beginning in 1999, MMTTs were performed routinely before and at 3, 6, 12, and 18 months, and OGTTs performed at 6 and 12 months, post-transplant (18). Subsequently, OGTTs were performed infrequently since patients disliked exposure to large glucose loads and generally only in insulin independent subjects. We analysed data from metabolic studies performed between 1999 and 2003 in subjects who had received their first islet infusion before 2002. The induction and maintenance immunosuppression received by subjects reflects the original Edmonton Protocol (daclizumab at induction and maintenance with sirolimus (8-10 ng/ml) and tacrolimus (4-6 ng/ml)).

Participants

Data from thirteen insulin-independent islet transplant recipients from Edmonton, with stable graft function, defined as capillary blood glucose readings <10mmol/L (tested 4 times per day over the previous 1 month), without exogenous insulin therapy or oral hypoglycemic agents with HbA1c <7% (53 mmol/mol) and normal renal function (eGFR>60ml/min) and who had undergone paired MMTTs and OGTTs are presented.

Metabolic studies

Three days before all studies, participants were asked to consume 250 g carbohydrates per day and abstain from alcohol or strenuous exercise, after which participants were studied in the Clinical Research Facility after an overnight fast of 8-

10 hours. The MMTT and OGTT tests took place on separate study days. The order of the paired tests were randomised.

Height was recorded to the nearest 0.5cm, weight to the nearest 0.1 kg (SECA 761 scales) and information regarding medications over the previous one week recorded. All participants had a 44-mm, 20-gauge cannula inserted in the left forearm for venous blood sampling. Participants acclimatised for 30 minutes prior to the ingestion of glucose or the mixed meal as previously described (27). The participant remained seated for the duration of the test.

Standardised MMTT: Sampling for glucose and C-peptide was done at baseline and 90-minutes after drinking Ensure HP (6ml/kg body weight to a maximum of 360 mls consumed within 5 minutes, providing 1.1 Calories/ml; 23% fat, 55% carbohydrate and 22% protein) (11, 27).

75g OGTT: Sampling for glucose, C-peptide and insulin were done at baseline and then following ingestion of 75g glucose at time 30, 60, 90 and 120 minutes.

All samples were centrifuged at 3000 rpm for 15 minutes at 4°C, separated and the plasma frozen at -70°C until analysis.

Biochemical assays were measured in duplicate and concentrations determined by the glucose oxidation method. C-peptide and insulin concentrations were measured using commercial kits (Roche Elecsys, Roche Diagnostics, Indianapolis, IN; and Pharmacia, Uppsala Sweden respectively) with lower limit of assay sensitivities of 0.02 nmol/L and 0.35 nmol/L respectively (27).

Ethical approval

Participants provided written informed consent and the study was approved by University of Alberta Health Research Ethics Board, and conducted in accordance with the principles endorsed by the Declaration of Helsinki and the Declaration of Istanbul.

Data Analysis

Measures of insulin resistance were derived from the HOMA-IR (28) and the Matsuda index (29) as measures of hepatic insulin resistance and whole body insulin sensitivity respectively (30). The insulinogenic index was derived as a surrogate measure of insulin secretion (31, 32) and the disposition index (DI) derived from the product of the Matsuda index and the insulinogenic index (30); a DI <1 is evidence of diminished insulin secretion in relation to the insulin sensitivity. Beta (27) and BETA-2 scores (11) were calculated as composite measures of graft function (See Supplemental Methods - Formulae of Scores).

Statistical analyses

Statistical analyses were performed using STATA version 15 (Stata Corporation, TX, USA). Descriptive statistics are expressed as mean±SEM and median(IQR) as appropriate. Data sets were compared using unpaired t tests, Mann Whitney U tests and ANOVA analyses with post-hoc testing. Comparisons between intra-individual OGTTs and MMTTs was compared by paired t-tests or Wilcoxon signed rank tests. The association between stimulated glucose values during the OGTT and MMTT were tested with Pearson correlation testing. The data sets were further compared without including the repeated measurements in the 4 participants. Area under the ROC (AUROC) curves were calculated with the 90-minute MMTT glucose concentrations according to $OGTT_{120}$ ≥11.1 mmol/L (200 mg/dL), the glycaemic threshold consistent with a diagnosis of diabetes (WHO criteria), to determine the accuracy of the test. The 90-minute MMTT glycaemic threshold corresponding with an $OGTT_{120}$ ≥11.1 mmol/L with maximum sensitivity and specificity was also derived from the ROC curve analyses. In post-hoc analyses, ROC curves of the MMTT data were constructed using a DI <1 and the specificity and sensitivity of the 90-minute MMTT-glycaemic thresholds generated in the primary analysis were derived.

Statistical significance was set at 5%.

Results

Seventeen paired OGTTs and MMTTs were performed in 13 participants; 9 participants received one OGTT and one MMTT; four participants received two OGTTs and MMTTs ((mean±SEM) 6±1 months between each paired study).

The time interval between first transplant and the metabolic test was (mean±SEM) 15±2 months. The paired MMTTs and OGTTs were performed a median of 2(1-2) days apart.

The personal data of the subjects is shown (Table 1). Subjects received an average of 2.1±0.1 transplants, with a mean time between the first and second transplant of 2.1±0.5 months. Mean creatinine of subjects was 80±3 umol/L, mean HbA1c 6.0±0.1%, beta scores 7.0±0 and BETA-2 scores 25±3 consistent with very good graft function.

The basal and stimulated glucose values pre and post the 75g OGTT and the MMTT at 120 minutes and 90 minutes respectively are shown (Figure 1A and 1B). Five studies had $OGTT_{120}$ ≥11.1 mmol/L. By WHO criteria, the remaining studies were classified as normal glucose tolerance (NGT) (n=5); isolated impaired glucose tolerance (IGT) (n=2); isolated IFG (n=1); or both IFG plus IGT (n=4). Of note only 2 of the 5 studies with $OGTT_{120}$ ≥11.1 mmol/L, had an HbA1c≥6.5% (48 mmol/mol) or fasting glucose ≥7.0mol/L. There was a positive correlation between the stimulated glucose values following the OGTT and the MMTT but this failed to reach statistical significance (r=+0.45; p=0.07).

Basal and stimulated C-peptide concentrations after OGTT and MMTT are presented in Tables 2A, 2B and 2C. The increment in C-peptide was greater after OGTT versus MMTT ($p<0.01$; Table 2C). However, the change in C-peptide in relation to the glucose concentrations, did not differ as evidenced by the AUC C-peptide in relation to the glucose concentrations (Table 2C).

When comparing studies with and without $OGTT_{120}$ ≥11.1 mmol/L there was no difference in HbA1c, beta or BETA-2 scores between the subgroups (Table 2A). Subgroup analyses of the four participants that were examined on two separate occasions did not demonstrate graft deterioration over time (BETA-2 scores and glucose and C-peptide concentrations during OGTT; all $p>0.05$). Of the n=4 participants tested on two occasions, one had $OGTT_{120}$ ≥11.1 mmol/L on both occasions.

During the OGTTs, studies with $OGTT_{120}$ ≥11.1 mmol/L versus those <11.1mmol/L had greater fasting and 2-hour glucose concentrations, greater glucose area under the curves (AUCs) and were relatively insulinopaenic with diminished 120-minute C-peptide: glucose concentrations, insulin: glucose and C-peptide: glucose AUC ratios from 0-120 mins (Table 2A). There was no significant difference in insulin sensitivity (HOMA-IR and Matsuda index). However insulin secretion (insulinogenic indices) and DIs were lower in the studies with $OGTT_{120}$ ≥11.1 mmol/L (Table 2A).

In contrast to the OGTT, there were no significant differences in the fasting and 90-minute glucose and C-peptide concentrations or in the 90-minute C-peptide: glucose concentrations; AUCs for glucose and C-peptide between 0-90-minutes; and the C-peptide: glucose ratio from 0-90-minutes during the MMTT between studies in those with or without $OGTT_{120}$ ≥11.1 mmol/L (Table 2B).

Individual glucose, C-peptide and insulin concentrations during the OGTT for each study are shown (Figure 1C-E). The 120-minute glucose and C-peptide concentrations following the 75g-OGTT were significantly greater than the 90-minute MMTT glucose and C-peptide concentrations (Figure 2A and 2B) although there was no difference in the stimulated C-peptide: glucose ratios (Figure 2C). Integrated

AUCs (0-90-minutes) for glucose and C-peptide were significantly greater following OGTTs versus the MMTTs ($p<0.001$; Table 2C).

When the analyses were repeated without the second observations in the 4 subjects, all results remained statistically significant.

The MMTT AUROC curves constructed with the 90-minute MMTT glucose according to $OGTT_{120}$ ≥11.1 mmol/L was 73±13%. A 90-minute MMTT glucose concentration of ≥8.0 mmol/L demonstrated 80% sensitivity and 83% specificity for $OGTT_{120}$ ≥11.1 mmol/L. In post-hoc analyses a 90-minute MMTT glucose ≥ 8.0 mmol/L was tested in diagnosing a DI <1, which in *a priori* analyses had been demonstrated to be significantly different in subjects with $OGTT_{120}$ ≥11.1 mmol/L versus $OGTT_{120}$ <11.1 mmol/L. Using the 90-minute MMTT glucose ≥ 8.0 mmol/L the AUROC for diagnosing a DI<1 was 77%.

Discussion

This study in insulin-independent islet transplant recipients with stable graft function and normal renal function, examined metabolic responses to a standardised oral glucose challenge and a mixed meal challenge. Not surprisingly there was a much greater increment in glucose after the larger glucose load of the OGTT, but both tests provided an equivalent estimate of graft function as judged by the integrated C-peptide in relation to the circulating glucose concentrations. Furthermore, we have shown that a 90-minute MMTT glucose ≥ 8.0 mmol/L is equivalent to a 2 hour glucose ≥11.1 mmol/L after a standard 75g-OGTT. Of note no participants were on exogenous insulin or oral hypoglycaemics and therefore there was no confounding of

metabolic results. Even in this highly selected cohort of narrow segment of subjects with insulin independent islet transplant recipients with very good graft function (shown by beta and BETA-2 scores), stimulation tests can identify individuals with sub-optimal graft function whose grafts may benefit from some metabolic support.

The MMTT is a valuable tool used to quantify graft function, rather than to identify glucose intolerance. Nevertheless, post challenge glucose concentrations were significantly higher after OGTT than post MMTT which is consistent with the MMTT being a more physiological and less potent stimulus of insulin secretion and therefore a less stringent challenge to graft function. While the post MMTT glucose levels were not able to reliably discriminate between those with and without $OGTT_{120}$ ≥11.1 mmol/L, both OGTT and MMTT were useful stimuli for insulin secretion measured by AUC for insulin and C-peptide. Such physiological testing with a standardised MMTT which contains less than half the amount of carbohydrate compared to a 75g-OGTT, avoids unnecessary hyperglycaemia therefore exposing the islet graft to less metabolic stress, is the preferred option to assess graft function following islet transplantation and is widely adopted by islet transplantation programmes world-wide permitting comparisons between subjects or within subjects over time (4, 27). Furthermore, such physiological testing has been adopted in new onset diabetes trials including TrialNet (19). A liquid meal is not truly physiologic however, and is associated with more rapid delivery of nutrients to the duodenum than after a solid meal (33). It does however avoid the confounding of delayed gastric emptying which might be anticipated in a cohort with long diabetes duration and high prevalence of diabetic neuropathy.

Raised fasting and post-prandial glucose are secondary to defects in either insulin secretion, insulin sensitivity, or both (34). The abnormal insulinogenic index and DI is consistent with defects in insulin secretion in these subjects with $OGTT_{120}$ ≥11.1 mmol/L post islet transplantation. Loss of the first phase insulin response with diminished suppression of hepatic glucose production, may be associated with fasting glucose concentrations as low as 5.0-5.4 mmol/L (35), and are well described in islet transplant recipients (10, 36) concordant with our observations where a high prevalence of impaired fasting glucose was seen. The mechanism is not known but diminished pulsatility of insulin secretion may play a role (37-42). Such studies underline the importance of studying islet transplant participants distinct from other groups with diabetes and extrapolations of data from other subjects with diabetes including those who are C-peptide positive, may be inappropriate (43, 44).

Of note the subjects selected for islet transplantation were insulin sensitive with normal body mass index (BMI) (6). Since insulin sensitivity was not measured by gold standard hyperinusulinaemic euglycaemic clamp studies, subtle defects in insulin sensitivity may have been missed. Certainly, immunosuppression with tacrolimus is recognised to induce insulin resistance and may contribute to insulin secretory deficits in some subjects; how this relates to the dose of immunosuppression as well as to their concentration is incompletely understood (45-47). In this study, subjects were receiving sirolimus and lower doses of tacrolimus (trough target 4-6 ng/ml) than is commonly employed when tacrolimus is combined with mycophenolate mofetil.

Studies that have examined subjects with Type 1 diabetes with paired OGTT and MMTTs have also demonstrated a 30% lower glucose concentrations with the latter test (48). Other studies comparing the standardised MMTT to a 75g-OGTT in subjects with a range of glucose tolerance who had repeated tests, demonstrated a linear correlation between the two tests at 120 minutes (49). This result concords with our observation of a positive association between the stimulated glucose values following the MMTT and the 75g OGTT, although in our study this just failed to meet statistical significance. The MMTT has been shown to be associated with lower glucose variability, fewer adverse symptoms and greater palatability versus the OGTT (49).

The primary analyses revealed a close relationship between the MMTT 90-minute glucose ≥8.0 mmol/L and glucose intolerance indicated by $OGTT_{120}$ ≥11.1 mmol/L. Since the objective of metabolic testing is to assess graft function post-hoc analyses were also performed to explore insulin secretion using ROC curves constructed using the DI<1, a composite score reflecting defective insulin secretion in relation to the insulin sensitivity (30, 50). Such analyses confirmed the close association of the 90-minute MMTT glucose with this further measure of graft dysfunction. Of note the HbA1c did not differ between islet transplant recipients with $OGTT_{120}$ ≥11.1 mmol/L versus those <11.1 mmol/L and therefore HbA1c was not examined further in secondary analyses. It is possible that recent onset of dysglycaemia could explain the similar HbA1c levels in subjects with and without diabetes, but more likely reflects the relatively low sensitivity of HbA1c for diagnosis of diabetes.

Of note variability in fasting plasma glucose on the days of the tests was observed. This did not reach statistical significance but intra-individual glycaemic variability of approximately 15% is well recognised even under standardised conditions as reported here (51).

Since a diagnosis of diabetes reflects inadequate insulin secretion to maintain euglycaemia these studies are important as they help identify a threshold where islet graft function may be sub-optimal. Although the MMTT does not clearly differentiate those with and without $OGTT_{120}$ ≥11.1 mmol/L, it does identify a corresponding threshold (90-min glucose ≥ 8mmol/L) associated with reduced DI. This threshold may identify subjects whose graft function should be monitored more closely; prompt efforts to minimise metabolic demands (perhaps through changes to nutrition and physical activity), or suggest consideration of therapies to support the graft or lower blood glucose levels (e.g. supplementary exogenous insulin, or other anti-hyperglycaemic agents). Delaying the institution of anti-hyperglycaemic therapies until overt hyperglycaemia, late in the spectrum of declining beta-cell function, will have limited ability to alter the natural history of beta-cell failure.

A limitation of the study is that glucose concentrations post MMTT were only measured at 90 minutes and not at 120 minutes or indeed earlier time points including 30 and 60 minutes. Since the peak glucose during a MMTT may occur at 30 or 60 minutes, using only a 0 to 90 minute may not accurately reflect the 'true' AUC, and may be an unfair comparison, side-by-side, to the OGTT AUC which has more values for calculation. Our comparisons of glycaemic indices with both tests at 90 minutes however concord with the literature (49) but comparisons of the MMTT and OGTT data at 120 minutes post-stimulus is not possible with our dataset. However, as most units perform glucose concentrations 90-minutes post MMTT in

islet transplant recipients, these results are of clinical utility. A further limitation of the study is the relatively small numbers of subjects studied. However, there are no other published studies comparing MMTTs and OGTTs in islet transplant recipients, and this study offers new insights in the field.

In summary we have studied a cohort of insulin independent subjects that have undergone islet transplantation for Type 1 diabetes with stable graft function and demonstrate a close association between a MMTT 90-minute glucose ≥8.0 mmol/L and the 120 minute OGTT threshold for the diagnosis of diabetes ($OGTT_{120}$ ≥11.1 mmol/L) (22). These studies reflect glycaemic thresholds during a MMTT that may identify individuals appropriate for intervention and intensification of efforts to support and protect graft function in the context of islet transplantation. Further studies are required to determine if early interventions alters the attrition in graft function seen post-transplantation and whether this in turn enhances the long-term benefits of islet transplantation to maintain good glycaemic control and protection from severe hypoglycaemia.

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Table 1. Personal Data of Subjects

Personal Data	**All**	**$OGTT_{120}$ <11.1**	**$OGTT_{120}$ ≥11.1**	**p**
Number of subjects	13	9	4	-
Number of paired OGTT and MMTT	17	12	5	-
Insulin independent (%)	100	100	100	-
Male: female	9:4	6:3	3:1	0.99
Age (years)	45.0±2.6	42.6±2.8	50.5±4.8	0.16
BMI (kg/m^2)	22.1±0.70	22.3±0.94	21.7±0.98	0.73
Pre-transplant dose of insulin (u/kg)	0.59±0.04	0.62±0.06	0.50±0.11	0.33
Time since transplant (months)	15.7±2.3	16.2±3.1	14.1±3.1	0.73
Number of transplants	2.1±0.1	2.0±0.2	2.2±0.2	0.52
IEQs	12,644 (10,858-15,709)	12,478 (10,858-13,723)	17,147 (10,752-17,147)	0.34
Duration of diabetes (years)	31±2.3	31.0±3.8	32.5±1.6	0.81
% retinopathy	62	49	75	0.58
% neuropathy*	54	33	75	0.21
% microalbuminuria	31	37	25	0.99
HbA1c	6.0±0.1	6.0±0.1	6.1±0.2	0.68
Beta score	7±0	7±0	6±0	0.36
BETA-2 score	25±3	25±3	25±3	0.96

Personal data in 13 islet transplant recipients undergoing n=17 paired OGTTs and MMTTs in study. Data shown is mean±SEM or median(IQR). p - statistical testing between islet transplant recipients with $OGTT_{120}$ <11.1 mmol/L versus $OGTT_{120}$ ≥11.1 mmol/L. *IEQ represents total number of islets received following transplants.**Neuropathy assessed clinically (autonomic and peripheral).

Table 2A. Metabolic data associated with 75g-OGTT

	All	$OGTT_{120}$ <11.1	$OGTT_{120}$ ≥11.1	p
Tests (n)	17	12	5	-
Fasting glucose (mmol/L)	6.1±0.1	5.7±0.1	6.8±0.2	0.002
120 min glucose (mmol/L)	9.7±0.7	8.2±0.6	13.4±0.5	0.0001
Glucose (AUC 0-120 mins) mmol/L.min	1116(934-1313)	1023(923-1162)	1368(1240-1516)	0.002
Fasting C-peptide (nmol/L)	0.67(0.58-0.83)	0.69(0.50-0.80)	0.65(0.58-0.76)	0.74
120 min C-peptide (nmol/L)	2.08(1.62-2.38)	2.16(2.02-2.38)	1.50(1.36-2.17)	0.29
C-peptide (AUC 0-120 mins)	205(170-212)	207(197-212)	185(170-230)	0.94
Fasting C-peptide: glucose	0.10(0.08-0.14)	0.11(0.08-0.14)	0.10(0.09-0.10)	0.90
120 min C-peptide: glucose	0.20(0.12-0.26)	0.24(0.2-0.35)	0.15(0.12-0.16)	0.04
C-peptide: Glucose (AUC 0-120 mins)	0.17(0.14-0.22)	0.21(0.18-0.25)	0.14(0.13-0.15)	0.01
Fasting insulin (mU/L)	8.5(5.5-16.7)	9.9(5.6-21.9)	8.5(5.3-13.9)	0.67
120 min insulin (mU/L)	34.2(29.3-45.4)	34.1(30.7-44.7)	35.7(25.3-55.0)	0.90
Insulin (AUC 0-120 mins) mU/L.min	2949(2543-5177)	3827(2711-5868)	2597(2255-3231)	0.12
Insulin: Glucose (AUC 0-120 mins)	2.9(1.9-4.5)	3.9(2.6-5.4)	1.9(1.8-2.0)	0.02
HOMA-IR	1.7±0.19	1.5±0.19	2.0±0.48	0.31
Matsuda Index	5.1±0.6	5.3±1.0	4.8±0.6	0.70
Insulinogenic index	0.40±0.06	0.43±0.06	0.14±0.01	0.004
DI	1.0(0.7-2.0)	1.66(1.01-3.45)	0.65(0.46-0.72)	0.005

Metabolic indices from OGTT (n=17 tests) in all studies and according to $OGTT_{120}$ <11.1 versus $OGTT_{120}$ ≥11.1. Data shown is mean±SEM or median(IQR). DI – disposition index; FPG – fasting plasma glucose, HOMA-IR – homeostatic modelling assessment-insulin resistance (C-peptide).
Matsuda index – measure of insulin sensitivity; insulinogenic index – measure of insulin secretion (22); DI –product of Matsuda index and insulinogenic index, <1 denotes relative insulin secretory defect (25). See Supplemental methods. Supplemental results show indices calculated at 90 minute time point and AUC 0-90 minute.
p - statistical testing between $OGTT_{120}$ <11.1 mmol/L versus $OGTT_{120}$ ≥11.1 mmol/L.

Table 2B. Metabolic data associated with MMTT

	All	$OGTT_{120}$ <11.1	$OGTT_{120}$ ≥11.1	p
N tests	17	12	5	
Fasting glucose (mmol/L)	6.4±0.2	6.3±0.2	6.6±0.3	0.38
90 min glucose (mmol/L)	7.8±0.6	7.2±0.7	9.0±0.9	0.16
Glucose (AUC 0-90 mins) mmol/L.min	617(542-745)	594(578-626)	711(648-779)	0.17
Fasting C-peptide (nmol/L)	0.72(0.56-0.92)	0.65(0.51-0.89)	0.81(0.72-1.03)	0.17
90 min C-peptide (nmol/L)	1.40(1.0-1.9)	1.33(1.01-1.58)	1.50(1.14-2.25)	0.65
C-peptide (AUC 0-90 mins) nmol/L.min	97(81-129)	91(80-105)	99(86-166)	0.27
Fasting C-peptide: glucose	0.11(0.10-0.14)	0.10(0.08-0.15)	0.13(0.11-0.14)	0.33
90 min C-peptide: glucose	0.21(0.16-0.25)	0.22(0.16-0.28)	0.17(0.16-0.21)	0.32
C-peptide: Glucose (AUC 0-90 mins)	0.16(0.13-0.20)	0.17(0.12-0.23)	0.15(0.13-0.18)	0.79

Metabolic Indices from MMTT (n=17 tests) in all studies and according to $OGTT_{120}$ <11.1 mmol/L versus $OGTT_{120}$ ≥11.1 mmol/L. Data shown is mean±SEM or median(IQR). p - statistical testing between $OGTT_{120}$ <11.1 mmol/L versus $OGTT_{120}$ ≥11.1 mmol/L.

Table 2C. Metabolic data associated with 75g-OGTT vs. MMTT

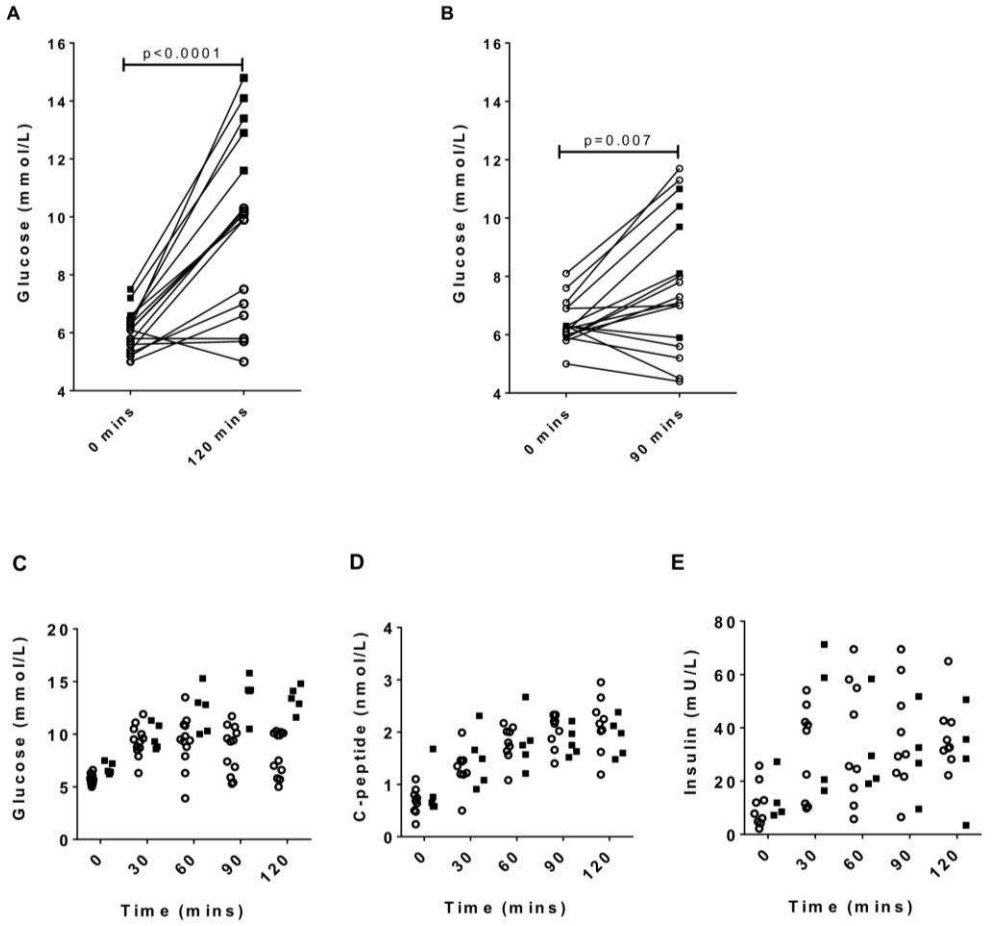
	OGTT	**MMTT**	**p**
Tests (n)	17	17	-
FPG (mmol/L)	6.1±0.1	6.4±0.2	0.07
Stimulated glucose (mmol/L)	9.7±0.7	7.8±0.6	0.01
Glucose (AUC) (mmol/L.min)	1116(934-1313)	617(542-745)	<0.001
Fasting C-peptide (nmol/L)	0.67(0.58-0.83)	0.72(0.56-0.92)	0.12
Stimulated C-peptide (nmol/L)	2.08(1.62-2.38)	1.40(1.0-1.9)	0.002
C-peptide (AUC) (nmol/L.min)	**205(166-215)**	**97(81-119)**	**<0.001**
Fasting C-peptide: glucose	0.10(0.08-0.14)	0.11(0.10-0.14)	0.08
Stimulated C-peptide: glucose	0.20(0.12-0.26)	0.21(0.16-0.25)	0.33
C-peptide: glucose (AUC)	0.17(0.14-0.22)	0.16(0.13-0.23)	0.85

Metabolic Indices from OGTT vs. MMTT in all participants.
Stimulated glucose and C-peptide measured at 120 mins post OGTT; 90 mins post MMTT.
AUC glucose and C-peptide measured between 0 to 120 mins post OGTT and from 0 to 90 mins post MMTT.

Figure 1. Glucose tolerance and metabolic profiles post islet transplantation.

Figure 2. Stimulated glucose (A), stimulated C-peptide (B) and stimulated C-peptide: glucose concentrations (C) following 75g-OGTT and MMTTs in islet transplant recipients.

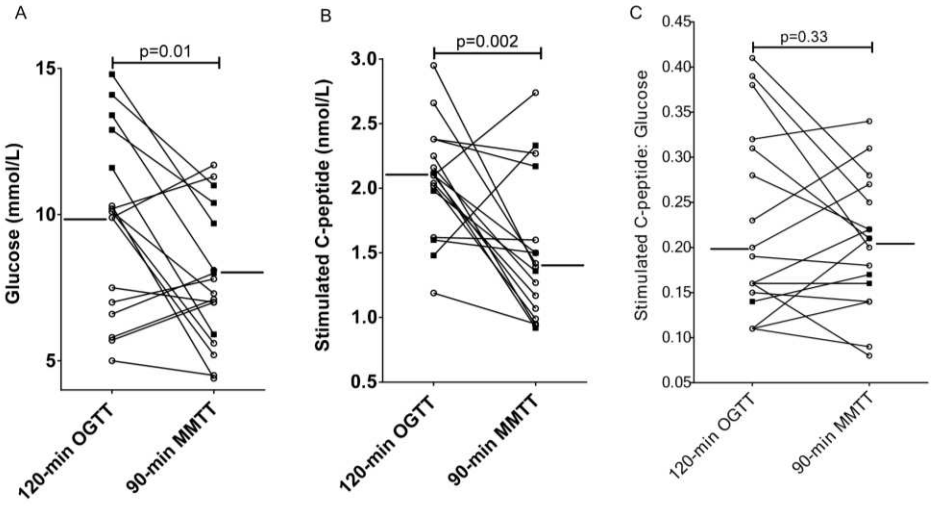
Figure 1. Glucose tolerance and metabolic profiles post islet transplantation.



A. Glucose concentrations at 0 and 120 mins post 75g OGTT in studies with $OGTT_{120}$ <11.1 mmol/L (n=12) versus $OGTT_{120}$ ≥11.1 mmol/L (n=5).
B. Glucose concentrations at 0 and 90 mins post MMTT in studies with $OGTT_{120}$ <11.1 mmol/L (n=12) versus $OGTT_{120}$ ≥11.1 mmol/L (n=5).
C. Glucose, D. C-peptide and E. Insulin concentrations during a 75g OGTT in n=17 studies post islet transplantation.

Open circles ($OGTT_{120}$ <11.1 mmol/L) versus filled squares ($OGTT_{120}$ ≥11.1 mmol/L).

Figure 2 Stimulated glucose (A), stimulated C-peptide (B) and stimulated C-peptide: glucose (C) concentrations following a 75g-OGTT and MMTTs in islet transplant recipients.



Glucose (A), stimulated C-peptide (B) and C-peptide:glucose concentrations (C) at 120-minutes following a 75g OGTT and corresponding metabolite concentrations at 90-mins post MMTT in n=17 studies post islet transplantation. Open circles ($OGTT_{120}$ <11.1 mmol/L) versus filled squares ($OGTT_{120}$ ≥11.1 mmol/L). Dashed lines – median values for subjects in tests.
Note paired metabolites compared at 90 minutes post OGTT vs. MMTT: glucose concentrations (p=0.004), stimulated C-peptide (p=0.003), stimulated C-peptide: glucose (p=0.60).