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BRIEF REPORT

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Donor insulin use predicts beta-cell function after islet transplantation

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Abstract

Insulin is routinely used to manage hyperglycaemia in organ donors and during the peri-transplant period in islet transplant recipients. However, it is unknown whether donor insulin use (DIU) predicts beta-cell dysfunction after islet transplantation. We reviewed data from the UK Transplant Registry and the UK Islet Transplant Consortium; all first-time transplants during 2008-2016 were included. Linear regression models determined associations between DIU, median and coefficient of variation (CV) peri-transplant glucose levels and 3-month islet graft function. In 91 islet cell transplant recipients, DIU was associated with lower islet function assessed by BETA-2 scores (β [SE] -3.5 [1.5], P = .02), higher 3-month post-transplant HbA1c levels (5.4 [2.6] mmol/mol, P = .04) and lower fasting C-peptide levels (−107.9 [46.1] pmol/l, P = .02). Glucose at 10 512 time points was recorded during the first 5 days peri-transplant: the median (IQR) daily glucose level was 7.9 (7.0-8.9) mmol/L and glucose CV was 28% (21%-35%). Neither

median glucose levels nor glucose CV predicted outcomes post-transplantation. Data on DIU predicts beta-cell dysfunction 3 months after islet transplantation and could help improve donor selection and transplant outcomes.

KEYWORDS
insulin, islet, organ donor, pancreas, transplant

1 | INTRODUCTION

In patients with type 1 diabetes mellitus, islet cell transplantation (ICT) provides an opportunity to alleviate life-threatening hypoglycaemia, improve glycaemic control, reduce the progression of diabetes-related microvascular complications and, in some cases, achieve insulin independence.[1,2] However, the routine attainment of insulin independence after islet transplantation from a single donor is unusual thereby prompting efforts to better understand donor selection and to improve post-transplant islet graft function.

Several factors, including higher donor glucose levels, are known to predict poorer outcomes after islet isolation.[3,4] Hyperglycaemia develops in ~ 50% of organ donors and is managed with insulin on intensive care units (ICUs). However, the relationship between donor insulin use (DIU) and outcomes post-transplantation has not been investigated. The co-administration of insulin and heparin during the peri-transplant period has been associated with higher rates of insulin independence post-transplantation.[5] However, there are limited observational data to suggest that tighter glycaemic control is associated with better beta-cell function post-transplant.

In this study, we first hypothesized that DIU predicts beta-cell dysfunction after islet transplantation and aimed to assess relationships of DIU with islet graft failure and function at 3 months after transplantation. Second, we hypothesized that tighter peri-transplant glycaemic control would be beneficial, and we aimed to determine the relationship between median levels and the co-efficient of variation (CV) of glucose levels during the peri-transplant period with measures of beta-cell function at 3 months after transplantation.

2 | METHODS

To assess relationships between DIU and islet transplant outcomes, the entire cohort of national data (2008-2016) from the UK Islet Transplant Consortium (UKITC) was reviewed. Our acceptance criteria for pancreas donors were: age 25-60 years, body mass index (BMI) 25-40 kg/m^2 and cold ischaemic time <12 hours. We excluded donors with known diabetes and HbA1c values of >48 mmol/mol (6.5%). Only first-time islet transplant recipients were included so that the relationships between DIU and glycaemic control during the engraftment period and outcomes post-transplantation could be accurately described without the potential influence of a prior islet cell transplant.

Peri-donation and peri-transplant glycaemic control

The UK NHS Blood and Transplant donor care bundle provides standardized guidance to maintain donor glucose levels at 4-10 mmol/L and, at blood glucose levels of >10 mmol/L, to commence a variable rate intravenous insulin infusion at a minimum rate of 1 IU/hour.[6] DIU was defined as any requirement for exogenous insulin during the peri-donation period. Data on donor insulin dosage are not routinely collected but dosage can be estimated from the duration of insulin treatment. Procurement of organs from donors in the UK follows a standardized operative procedure in keeping with guidance from NHS Blood and Transplant.[7] Glucose levels during the first 5 days post-transplantation were obtained from capillary blood glucose and continuous glucose monitoring data. The peri-transplant target blood glucose range was 4.0-6.9 mmol/L, and an intensive variable rate intravenous insulin therapy regimen was followed for the first 48 hours, and subcutaneous or pump insulin therapy thereafter.

2.1 | Islet, isolation, assessment and transplantation

Pancreases were transported at 4°C in University of Wisconsin solution to one of three human islet isolation facilities located in Edinburgh, London or Oxford. Following digestion with collagenase NB 6 GMP grade (Serva, Heidelberg, Germany) and automated mechanical dissociation in a Ricordi chamber, islets were purified on a continuous density gradient using a COBE 2991 processor. Islets were cultured for a minimum of 24 hours and released for transplantation if the following criteria were met: islet mass >3000 IEQ/kg, viability >70% and purity >50%. When the allocated recipient was located at a different unit to the isolation centre, islets were resuspended in 500 mL of CMRL medium (PAA Laboratories, Somerset, UK) supplemented with 2% human serum albumin (ZENLAB20; Bio Products Laboratories, UK) and 2 mM HEPES (PAA Laboratories) then transported by road in cooled standard blood transfusion bags. Islets were transplanted by radiological guided percutaneous trans-hepatic infusion into the portal vein.

2.2 | Defining graft function and failure

Graft function was determined by HbA1c, fasting and stimulated glucose and C-peptide levels, and the BETA-2 score assessed at 3 months post-transplantation. The BETA-2 score is a composite measure of graft function including HbA1c, fasting and 90-minute stimulated C-peptide and glucose levels, and insulin requirement.[8] Graft failure was defined as stimulated C-peptide of <50 pmol/L.

2.3 | Analysis

We assessed the distributions of donor and recipient variables for exposures, covariates and outcomes. Univariable and multivariable adjusted linear regression models explored relationships between DIU status and graft function. Multivariable adjusted models included covariates identified through univariable associations with DIU status, and also included covariates widely reported as potential confounders. Linear regression related covariates to median glucose levels and the glucose CV. Univariable and multivariable linear regression models related median glucose levels and glucose CV to graft function outcomes outlined above.

3 | RESULTS

Of the 91 first-time ICT recipients (female: n = 40 [44%]; mean [SD] age 50 [10] years), 52 (57%) had a donor treated with insulin on ICUs. The median (IQR) islet yield was 390 (320-495) x 1000 IEQ and median viability was 90% (85%-90%) and purity 75% (70%-85%). All patients received immunosuppression with tacrolimus and mycophenolate, 10 of whom also received steroids (in the early years of the programme). Induction therapy with alemtuzumab (Campath) was given routinely to all patients apart from eight (9%) who received basiliximab (Simulect), and one (1%) who received rabbit antithymocyte globulin. Seven (8%) patients also received anti-TNF therapy (etanercept).

DIU was not significantly associated with any measured donor characteristics that could confound relationships between DIU and graft outcomes (Table 1). Statistically significant relationships were

TABLE 1 Donor characteristics associated with donor insulin use on intensive care in islet cell transplantation

Donor variables	Donor insulin use in intensive care		P-value
	Yes (n = 52)	No (n = 39)	
Age, years	46 (10)	45 (10)	.80
BMI, kg/m^2	29 (10)	29.3 (5)	.48
Sex (female)	28 (54)	26 (67)	.53
Ethnicity			
White	45 (87)	36 (92)	.51[a]
Smoking	22 (43)	15 (40)	.83
Alcohol	5 (10)	2 (5)	1.00
Hypertension	8 (16)	10 (26)	.13
Cardiac disease	2 (4)	5 (13)	.23
Cardiac arrest	12 (24)	10 (26)	1.00
Peri-retrieval hypotension	37 (71)	26 (67)	.66
Donor type (DBD)	46 (89)	33 (85)	.76
Cause of death			
Trauma	4 (8)	5 (13)	.49
Meningitis	1 (2)	0 (0)	NA
Stroke (thrombo-embolic)	1 (2)	2 (5)	NA
ICH	31 (60)	24 (62)	1.00
HBI	9 (17)	3 (8)	.22
Brain tumour	2 (4)	1 (3)	NA
Other	4 (8)	4 (10)	NA
Amylase, U/l	56 (34-115)	54 (37-100)	.89
Steroid use	25 (61.0)	33 (64)	.83
Islet yield (IEQ/kg)	6193 (2122)	5822 (1695)	.42
HbA1c (mmol/mol)	37 (5.2)	35 (3.2)	.45

Abbreviations: BMI, body mass index; DBD, donation after brain death; ICH, intracranial haemorrhage; HBI, hypoxic brain injury; NA, not applicable.

[a]White versus non-white ethnicity. Data are mean ± SD or n (%) or median (IQR).

shown between DIU and higher 3-month post-transplant HbA1c (mean [SD] HbA1c, DIU vs. non-DIU: 50 [2.0] vs. 45 [1.5] mmol/mol, $P = .04$), higher fasting and 90-minute stimulated glucose (fasting: 9.7 [0.8] vs. 7.8 [0.5] mmol/L, $P = .04$ and 90 minute: 15.8 [1.0] vs. 12.9 [0.7] mmol/L, $P = .024$); lower fasting C-peptide (191 [20.7] vs. 299 [43.9] pmol/L, $P = .02$) and a lower BETA-2 score (7 [0.9] vs. 11 [1.2]), $P = .02$). These relationships remained significant after adjusting for age, BMI and the total number of islets transplanted (IEQ/kg) (Table 2). Rates of graft failure were higher in transplants from DIU compared with non-DIU although this was not statistically significant (5/45 vs. 1/41, $P = .22$). In DIU, duration of insulin was not significantly associated with any outcomes.

Complete data on peri-transplant glucose levels were available from 69 islet transplant recipients. We recorded 10 512 glucose levels (68 [53-88] per patient) during the first 5 days post-transplant. The median (IQR) daily glucose level was 7.9 (7.0-8.9) mmol/L and glucose CV was 28% (21%-35%). DIU (β [SE] 1.17 [0.67], $P = .09$) was identified as a nominal covariate of median daily glucose levels, while donor BMI (−0.66 [0.35], $P = .06$) was a nominal covariate of glucose CV (Table S1). Neither median daily glucose nor the CV of glucose levels were significantly related to any measures of graft function at 3 months after islet transplantation (Table S2).

TABLE 2 Univariable and multivariable-adjusted β-coefficients (SE) relating donor insulin use with 3-month HbA1c, 90-minute glucose and 90-minute C-peptide during meal tolerance testing in islet transplant recipients

Measure of graft function	β coefficient (SE)	*P*-value
Unadjusted		
HbA1c (mmol/mol)	5.36 (2.62)	.04
Fasting glucose (mmol/L)	1.96 (0.92)	.03
90-min glucose (mmol/L)	2.84 (1.23)	.02
Fasting C-peptide (pmol/L)	−107.90 (46.10)	.02
90-min C-peptide (pmol/L)	−145.00 (106.80)	.18
BETA-2 score	−3.53 (1.50)	.02
Insulin reduction	−0.04 (0.03)	.21
Adjusted for age, BMI and islet yield[a]		
HbA1c (mmol/Mol)	7.05 (3.08)	.04
Fasting glucose (mmol/L)	2.44 (0.93)	.02
90-min glucose (mmol/L)	3.85 (1.40)	<.01
Fasting C-peptide (pmol/L)	−100.24 (50.10)	.05
90-min C-peptide (pmol/L)	−117.91 (104.46)	.26
BETA-2 score	−3.87 (1.64)	.02
Insulin reduction	−5.46 (7.58)	.47

Abbreviation: BMI, body mass index. The BETA-2 score[8] is a composite measure of graft function which includes the following data: fasting glucose, fasting C-peptide, HbA1c and insulin dose. The β coefficients associated with donor insulin use (DIU) can be interpreted as the average difference in the outcome (e.g. HbA1c level) between donors treated with insulin and donors not treated with insulin. Positive values indicate higher levels in the DIU group compared with the non-DIU group.

[a]Covariates identified in the published literature.

4 | DISCUSSION

4.1 | Main findings

We have shown that DIU is associated with poorer graft function in ICT recipients. DIU was associated with: (1) a lower BETA-2 score 3 months post-ICT; (2) a higher HbA1c 3 months post-ICT; (3) a higher fasting and 90-minute glucose level 3 months post-ICT; and (4) a lower fasting C-peptide 3 months post-ICT. These data are consistent with our hypothesis that DIU is a predictor of adverse outcomes in islet transplantation.

4.2 | Prior studies

To the best of our knowledge, this is the first study assessing relationships between DIU and peri-transplant glycaemic control and measures of graft function in islet cell transplant recipients. Two relevant studies evaluated the relationship between donor hyperglycaemia and the success of human islet isolation and reported conflicting findings.[3,9] In the smaller study there was no relationship between donor hyperglycaemia or insulin use, and success from islet isolation was attributed to a higher islet yield in the successful preparations.[9] A larger study involving 153 islet isolations reported an association between lower donor blood glucose levels and successful islet isolation[3] and led to the subsequent inclusion of blood glucose levels in a composite scoring system to assess donor pancreases.[4]

4.3 | Mechanistic insights

We showed that ~ 50% of organ donors require exogenous insulin on ICUs because of hyperglycaemia. DIU could simply be a marker of donor insulin resistance, which is well documented after brain death, and could be exacerbated by inflammation, inotrope and corticosteroid use, and low cardiac output leading to muscle and liver hypoperfusion.[10–12] Theoretically, these scenarios leading to beta-cell stress would be reversible at the time of organ retrieval when the pancreas is removed from the stressors causing donor insulin resistance. In our data, similar HbA1c levels in both the DIU and non-DIU groups suggest that DIU does not occur because of pre-existing underlying defects in beta-cell function or mass. It seems plausible that hyperglycaemia leading to DIU on ICUs occurs as a consequence of a combination of beta-cell stress (donor insulin resistance) and beta-cell death. The alternative scenario that DIU could be explained by beta-cell death requires further research.

Insulin therapy to treat hyperglycaemia also promotes beta-cell rest.[13] Therefore, it is possible that DIU may have a beneficial effect, especially in donors experiencing severe beta-cell stress. Donors with untreated hyperglycaemia may represent the phenotype at highest risk of graft failure.

4.4 | Clinical implications

Clinicians should be aware that DIU predicts beta-cell dysfunction 3 months after islet transplantation. This could help to appropriately

discard donor organs at higher risk of failure and expand the donor pool by using organs not exposed to insulin previously considered unsuitable based on donor characteristics alone.

Based on our data, as well as from previous studies, the optimum peri-transplant target glucose range is unclear.[5] In light of our results, we do not support the pursuit of tighter glycaemic control during the peri-transplant period, nor do we advocate widening the current target glucose range and loosening of glycaemic control.

4.5 | Strengths and limitations

Our study has several strengths. First, using data from the entire UK cohort of islet cell transplantation, we provide the first description of the relationship between DIU and clinical outcomes. Second, we have provided further evidence to support the notion that data on insulin use are a better determinant of outcomes than glucose levels alone.

We acknowledge some limitations: first, we have a modest number of beta-cell function outcomes, which leads to wide confidence intervals around risk estimates and a limited ability to adjust for potential confounders. Second, our data do not exclude the possibility that insulin therapy on ICUs is harmful to donor pancreases. However, this hypothesis seems unlikely because of the known beneficial effects of insulin on beta cells[13] and because high glucose levels occur on ICUs before and not after insulin is prescribed. Third, accurate data on donor insulin dosage are not routinely collected. While we could not assess the precise relationship between insulin dosage and outcomes, data on insulin duration provided an estimated relationship with dosage. Finally, follow-up was limited to 3 months because a second islet infusion from a different donor, routinely occurring at 3-6 months, means that outcomes thereafter cannot be attributed to individual donor factors.

CONCLUSION

In conclusion, we provide data supporting the hypothesis that DIU is a predictor of beta-cell dysfunction in islet transplant recipients. Further effort is required to clarify the underlying mechanisms that might lead to better transplant outcomes in islet donors and recipients receiving insulin therapy: these mechanisms can then be harnessed and applied in the clinical setting as part of the broader aim of achieving long-term insulin independence after islet transplantation from a single donor. Identifying a threshold for blood glucose levels or insulin dose requirement associated with a poorer outcome could help to exclude non-suitable donors.

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CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

I.M.S. – all aspects of the study, lead author. A.S. – all aspects of the study, but not data collection. P.Y. – data collection, data interpretation, writing. H.K. – data collection, data interpretation, writing. C.F. – data analysis, statistical oversight, writing. N.A.H. – study concept and oversight, writing. J.C. – Chair of Pancreas Advisory Group at NHS Blood and Transplant with oversight over national data collection; writing. S.F. – Principal Investigator (Edinburgh); writing. M.R. – Principal Investigator (Royal Free, London); writing. P.R.V.J. – Principal Investigator (Oxford); writing. P.C. – Principal Investigator (Kings College, London); writing. J.B. – Principal Investigator (Bristol); writing. J.A.M.S. – Chair of the UK Islet Transplant Consortium; Principal Investigator (Newcastle); writing. T.A. – Deputy Chair of Pancreas Advisory Group at NHS Blood and Transplant with oversight over national data collection; study concept and oversight; writing. M.K.R. – all aspects of the study, senior author. D.v.D. – all aspects of the study, senior author.

ETHICS STATEMENT

All data were collected prospectively, with ethical approval (UKITC 07/Q0904/11), and with written informed consent from all donor families and transplant recipients.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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