The BETA-2 Score: a Tool for Assessing Beta Cell Function in Clinical Islet Transplantation and Type 1 Diabetes Intervention Trials

by

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ABSTRACT

Preservation of beta cell function is integral to clinical islet transplantation (CIT) and in the development of treatments which halt the progression of type 1 diabetes (T1D). Outcome assessment remains difficult in both fields as the preservation of beta cell function and maintenance of glucose homeostasis must be considered. Stimulated C-peptide is the gold standard for measuring beta cell function, but it is time and labor intensive to measure. Insulin use and measures of glycemic control are inter-dependent and alone are unlikely to capture changes in beta cell function. The BETA-2 score was developed in CIT as a practical measure of beta cell function that also provides information on overall metabolic status. I demonstrate here the utility of the BETA-2 score in assessing islet engraftment in CIT. I also explore the use of the BETA-2 score as a clinical endpoint in T1D intervention trials.

PREFACE

Parts of the introduction were written in collaboration with Dr. Richard A. Oram as a part of an invited review on persistent C-peptide for the journal Current Diabetes Reports.

Chapter 2 of this thesis is prepared for submission as: Anna Lam MD, Richard A Oram PhD, Shareen Forbes PhD,Tolu Olateju MBBS, Sharleen Imes MSc, Andrew J Malcolm PhD, AM James Shapiro PhD, Peter A Senior PhD. Early Islet Engraftment in Clinical Islet Transplantation Can be Assessed Using BETA-2 Score and Predicts Outcome at 2 years. A.L and P.S. drafted the manuscript and analyzed and interpreted the data. A.L and S.I researched the data. All authors contributed to revision of the article and approved the final version of the article.

Chapter 3 of this thesis contains research that was done in collaboration with Dr. Richard Oram and Dr. Robert Andrews at the University of Exeter, Dr. Michael Haller at the University of Florida, and Dr. Peter Senior at the University of Alberta. Data were provided by Dr. Andrews and Dr. Haller. I was involved in the data analysis and drafting of the chapter as presented here.

DEDICATION

This thesis work is dedicated to my mom a.k.a Big Pam.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1 : Introduction

Type 1 diabetes (T1D) is a chronic autoimmune disease that results in progressive loss of beta cell mass. In the classic model of T1D, overt diabetes is only observed after there is extensive beta cell loss with the disease ending finally in complete beta cell destruction (1). However, while T1D is still widely viewed as a state of absolute beta cell deficiency, there is mounting evidence that the disease course is likely more complex (2-4). Here we discuss evidence supporting the persistence of beta cells in T1D. We also discuss the challenges of evaluating beta cell function and establishing its clinical relevance in type 1 diabetes intervention trials and clinical islet transplantation (CIT).

Persistence of beta cell in type 1 diabetes

A historical lack of human samples and reliance on animal models has contributed to the long held view of absolute beta cell destruction in T1D. Non-obese diabetic (NOD) mice share many genetic and immunologic similarities with human T1D and has therefore been a favored model of study (5) . However, differences in disease pathogenesis do exist, and these may explain, in part, why the majority of treatments which prevent beta cell loss in NOD mice have been ineffective in humans (6). Notably, NOD mice show a phenotype of severe insulitis and beta cell destruction. Conversely, severe insulitis is rarely observed in humans (7, 8) and there is evidence of persistent beta cells at various stages of human T1D (4, 9-12). More recently, studies done on human samples have revealed heterogeneity in insulitic and inflammatory phenotypes, which cannot be appreciated in animal models (13-

16). Initiatives, such as the Network of Pancreatic Organ Donor with Diabetes (nPOD), which recover and provide human pancreatic tissue for study are therefore critical in providing a better understanding of the persistence of beta cells in T1D (17).

Persistence of C-peptide in type 1 diabetes

Initially, studies showing persistent beta cells in human T1D were dismissed as these cells were deemed non-functional and of little clinical consequence (9). However, for as long as C-peptide has been measured, there has been evidence of persistent beta cell function in T1D (18-23) .

C-peptide is released from the cleavage of proinsulin and secreted into the portal circulation in equimolar concentrations to insulin (Figure 1.1) (24). It is a better measure of endogenous beta cell function than insulin, given that it does not undergo hepatic extraction and therefore has a longer half-life and it avoids the risk of assays which measure both endogenous and exogenous insulin. Measurement of stimulated C-peptide following a mixed meal tolerance test (MMTT) provides a standardized and sensitive measure of beta cell function and is generally used in T1D intervention trials and clinical islet transplantation (25). C-peptide can also be measured in the fasting state or post-prandially. Ultrasensitive assays of C-peptide are also available and have detection limits as low as 1.5 pmol/L compared to conventional limits of 30 pmol/L (26).

Recent studies have demonstrated a wide range of persistent C-peptide in T1D including very low to normal fasting C-peptide levels measured within the first year of diagnosis in

diabetic youths (27). It has also been suggested that nearly 1 out 3 T1D patients >3 years from diagnosis have measurable non-fasted C-peptide (28). Impressively, persistent C-peptide has been demonstrated in patients with very long duration of diabetes; the Joslin medalist study which included patients with ≥ 50 years duration of disease found detectable C-peptide (>0.03 nmol/L) in 67.4% of participants and C-peptide ≥0.2 nmol/L in 2.6% of participants (4). More recently, studies using ultrasensitive C-peptide assays have demonstrated that the majority of patients with long duration T1D secrete very low levels of C-peptide and are so called "microsecretors" of C-peptide (29-31).

Clinical relevance of persistent C-peptide

Anecdotally, the benefit of persistent C-peptide has long been reported by physicians and patients who intuitively attribute the near-normal glucose levels and reduced insulin requirements observed during the honeymoon period to transient maintenance and/or restoration of beta cell function. However, it was not until the Diabetes Control and Complications Trial (DCCT) that the concept of clinical benefit associated with persistent C-peptide came to the fore. Although the DCCT is best known for establishing the importance of intensive glycemic control in T1D, it also remains one of the largest studies of C-peptide in T1D patients (32). A total of 3763 patients were screened prior to study enrolment and among patients diagnosed with T1D after the age of 18**,** stimulated C-peptide was >0.2 nmol/L in 48% of patients with T1D duration of 1-5 years and 8% among those with T1D duration 5-15 years (33-35). Importantly, these patients were found to have better glycemic control, with lower fasting blood glucose and hemoglobin A1C (A1C). They also had reduced risk of hypoglycemia and retinopathy progression. Based on these

results, a stimulated C-peptide >0.2 nmol/L is generally regarded as being clinically relevant. It is important to note, however, that further studies are required to substantiate these results. Furthermore, a causal relationship between persistent C-peptide and clinical benefit has not yet been established. Lastly, it remains unknown whether there is any clinical benefit associated with the very low C-peptide levels detected by ultrasensitive assays.

Preserving C-peptide in the treatment of T1D

The persistence of C-peptide in T1D presents an opportunity to preserve and/or optimize beta cell mass and function perhaps even long after diagnosis. Thus far, intervention trials have primarily focused on early disease (at or shortly after diagnosis). Although some treatments have shown promise, their effects have been inconsistent or are associated with toxicities that preclude their clinical use (36). Several issues have been identified as contributing factors to the largely disappointing results from T1D intervention trials. These include the development of treatments in animal models which do not reflect human T1D (as detailed above), the use of single rather than combinations of immune-modulating treatments, and various issues in trial design, including the choice of trial endpoints (36).

Endpoints in T1D interventions trials

An ongoing issue in T1D intervention trials is defining appropriate endpoints (37). Stimulated C-peptide measured following MMTT is accepted as the most appropriate primary outcome. (25). However, persistent C-peptide is only useful to the extent that it provides clinical benefit. As such, a treatment which effectively preserves C-peptide should

also be shown to be associated with clinical benefit. A practical consideration is the time- and labor-intensive nature of MMTTs which are inconvenient for patients and add to trial costs.

Glycated hemoglobin, fasting blood glucose and insulin use are clinically relevant and practical endpoints to be considered. However, as individual outcomes, they are likely insufficient to detect treatment responses (38). Furthermore, measures of glycemic control may be insensitive to treatment effect given that treated and control subjects share the same glycemic targets. Thus, having a clinical score which incorporates various measures of glycemic control, insulin use and beta cell function would be useful as a practical outcome in T1D intervention trials.

Parallels between T1D intervention trials and clinical islet transplantation

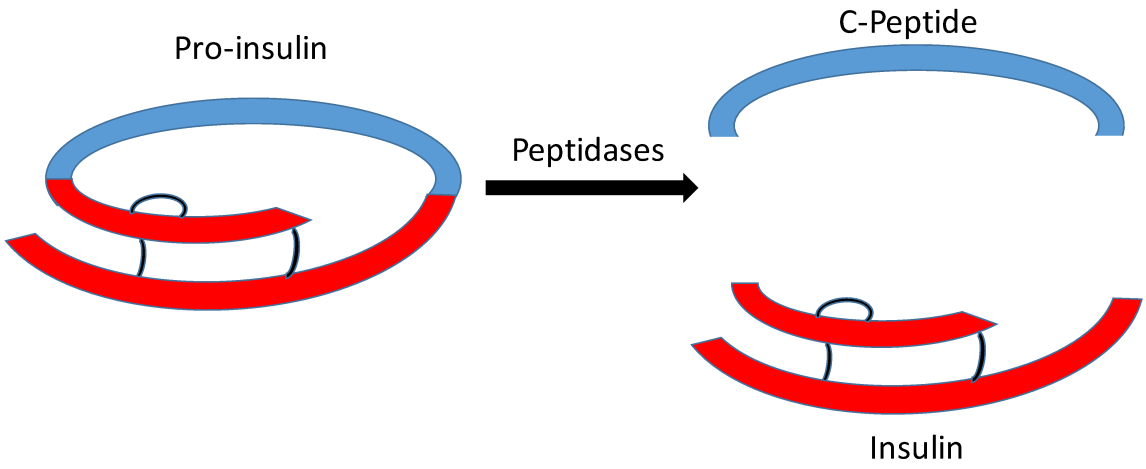
The benefits of restoring endogenous insulin production have been well established in CIT. Outcomes following CIT vary depending on the level of graft function achieved; patients with optimal graft function achieve near normal glucose control off insulin, while patients with relatively minimal graft function may also benefit from improved glycemic homeostasis, protection from hypoglycemia, and reduced insulin requirements (38-41). As preservation of beta cell function is also central to CIT, the challenges that exist in T1D trials in terms of defining appropriate endpoints also apply in CIT. To address these issues, the BETA-2 score was developed as a simple method for assessing both beta cell function and overall metabolic status in CIT.

The BETA-2 Score in CIT and T1D intervention trials

The BETA-2 score has been validated as an index of beta cell function in CIT. It is calculated from a single blood sample and includes fasting C-peptide, fasting blood glucose, A1C and insulin dose (42). It is easily interpretable and has been shown to detect clinically relevant scenarios in CIT (42). Taken together, the BETA-2 appears to be an ideal tool for monitoring patients in CIT and potentially as an endpoint in T1D intervention trials.

In the current study, I evaluate the BETA-2 score as an outcome in CIT and T1D intervention trials. In the first part of the study, the aim was to demonstrate the clinical utility of the BETA-2 score in monitoring islet engraftment in the first 6 months post-CIT. In the second part, the aim was to retrospectively evaluate the BETA-2 score as an outcome in two previously completed pilot T1D intervention trials.

Figure 1.1 Pro-Insulin is cleaved to form free C-peptide and insulin.



Adapted from Kitabchi AE. Proinsulin and C-peptide: a review. Metabolism. 1977 ;26(5):547-87.

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Chapter 2 : Early Islet Engraftment in Clinical Islet Transplantation Can be Assessed Using the BETA-2 score and Predicts Outcome at 2 years

INTRODUCTION

Clinical islet transplantation (CIT) is an established treatment in select type 1 diabetic patients who have severe recurrent hypoglycemia and glycemic lability despite optimized medical therapy. Long term outcomes post-CIT are highly variable with some patients maintaining insulin independence, while others need to resume insulin, but at reduced doses with improved glycemic homeostasis and protection from hypoglycemia (CIT phase 3 trial) (1) .

Optimizing islet engraftment is a potential target in improving CIT outcomes (2, 3). Indeed, optimal primary islet graft function in the early period post CIT has been shown to be associated with prolonged graft survival and better metabolic control (4). Unfortunately, although several strategies for improving islet engraftment have shown promise in animal models, few have translated to clinical success. Furthermore, little is known about islet engraftment in the clinical setting; few studies have described islet engraftment and those that are available have been limited to the first hours to days post-CIT (5, 6). One of the major barriers in this area remains the difficulty in closely monitoring graft function post-transplant.

Formal stimulation tests measuring insulin or C-peptide response to stimuli such as glucose or arginine can provide precise information on graft function, however, their results can vary depending on the protocol used and may be discordant (i.e. good response to mixed meal tolerance test (MMTT) but minimal response to arginine) (7). Additionally, the time and labor intensive nature of stimulation tests limits their use in routine clinical practice (8). The glucose potentiated arginine test is viewed as the most precise tool to assess functional beta cell mass (9), but is particularly time consuming and its use is restricted to research settings. Fasting C-peptide and blood glucose levels are alternative measures of graft function that can be easily measured. However, they are highly variable and inter-dependent and are limited in their ability to capture changes in graft function if considered individually.

The BETA-2 score is a novel and validated composite index of islet graft function that integrates glycemic control (hemoglobin A1c (HbA1c)), insulin use and paired fasting C-peptide and glucose concentrations (10). It is a refinement of the original beta-score, which is an established index of islet graft function (4, 11, 12). The BETA-2 score has been shown to better detect insulin independence compared to the original beta-score (10). Moreover, the BETA-2 score can be easily calculated from a single fasting blood sample, unlike the original beta-score, which generally required a mixed meal test to measure stimulated C-peptide (11). As a result, the BETA-2 score can be monitored on a regular and frequent basis. Taking advantage of the fact that the BETA-2 score can be calculated from a single blood sample, we estimated islet graft function on a weekly basis following CIT to assess islet engraftment.

The aim of this study was to demonstrate the clinical utility of the BETA-2 score in monitoring islet engraftment by comparing the time course of islet engraftment over the first 6 months post-CIT in subjects achieving insulin independence at 12 months after a single transplant compared with subjects requiring a second islet transplant. We also explored whether early islet engraftment was associated with longer term insulin independence.

METHODS

Patients

We performed a retrospective single center analysis of type 1 diabetes participants newly transplanted with allogeneic islets between 2009 and 2014. All subjects provided informed consent, and the analysis of data was approved by the University of Alberta Health Research Ethics Board.

Two groups of subjects were identified and included for analysis: group 1CIT subjects achieved insulin independence for at least 12 months after a single islet transplant (1CIT) and group 2CIT subjects who required a second islet transplant (2CIT) 3-6 months following 1CIT before achieving insulin independence for at least 1 month. Insulin independence was defined by the use of no exogenous insulin for 30 days and no more than 2 self-monitored blood glucose levels >10.0 mmol/L. Subjects enrolled in the Collaborative Islet Transplant consortium trial or subjects receiving more than two islet

transplants were not included. We excluded subjects receiving a second islet transplant within 3 months to clearly differentiate the engraftment of two transplants, and those waiting more than 6 months for a second transplant in order to limit other variables (i.e. high BMI, or elevated panel reactive antibodies (PRA)), which may have confounded the timing of second transplant and also introduced increased variability in graft function between subjects.

All subjects were C-peptide negative before islet transplantation. The indications for islet transplantation, islet preparation, transplant procedure and monitoring have been previously described (13, 14). Immunosuppression consisted of induction with lymphodepleting antibodies (alemtuzumab or thymoglobulin) and maintenance with tacrolimus (target trough levels 8-10 ng/ml) and mycophenolate mofetil (1g bid as tolerated).

Clinical assessment

Severity of hypoglycemia and glycemic lability were assessed pre-transplant by the hypoglycemic score (HYPO score) and lability index (LI), respectively (15). Subjects were seen weekly in clinic during the first month post-transplant and then every 3-6 months in the first year post transplant. Subjects were asked to self-monitor blood glucose and insulin usage. Insulin dose (U/kg) was calculated based on reported insulin dose divided by body weight measured at the most recent clinical assessment. Blood work including fasting C-peptide, fasting glucose, and tacrolimus level were measured every 1-2 weeks during the

first 6 months post-transplant. HbA1c (as a percentage) was measured every 1-3 months post-transplant.

Assays

Fasting plasma glucose concentrations were determined by the glucose oxidase method. C-peptide concentrations were measured using a commercial assay (Roche Elecsys; Roche Diagnostics, Indianapolis, IN). The lower limit of sensitivity for C-peptide in our laboratory was 0.02 nmol/L and the inter-assay coefficient of variation was 3.5%. HbA1c was measured by the Bio-Rad Variant II kit (Hercules, CA). Screening for PRA was initially performed by Luminex® or FlowPRA® method and further testing for antibody specificities was done if the screen was positive.

Calculation of BETA-2 score

Weekly BETA-2 scores were calculated post-CIT. Derivation and validation of the BETA-2 score have previously been described (10). The BETA-2 is generated based on fasting C-peptide (nmol/L), daily insulin dose (units/kg), fasting plasma glucose (mmol/L) and HbA1c (%) as follows:

$$\text{BETA-2 Score} = \frac{\sqrt{(\text{fasting C-peptide}) \times (1 - \text{insulin dose})}}{\text{fasting plasma glucose} \times \text{HbA1c}} \times 1000$$

Statistics

Statistical analyses were performed using Stata version 14.1 (StataCorp, College Station, TX). Descriptive statistics are expressed as mean $\pm$ standard deviation (SD). Two-tailed t-

test and Fisher's exact test were used to compare groups as appropriate. Associations between variables were estimated by simple linear regression. A P value of less than 0.05 was considered statistically significant and all P values were reported as two-sided.

RESULTS

Patient characteristics

Between 2009 and 2014, a total of 79 patients were newly transplanted with allogeneic islets. Fifteen subjects were included in the current analysis (group 1CIT, n=8 and group 2CIT, n=7). Excluded patients included subjects who did not achieve insulin independence after islet transplantation (n=5), subjects who achieved insulin independence after a single islet transplant, but for less than 1 year (n=11), and those who received a second islet transplant less than 3 months or more than 6 months after initial transplant (n=48).

Group 1CIT subjects achieved and maintained insulin independence for 3.9 $\pm$ 1.9 years after 1CIT compared to group 2CIT subjects who received their 2CIT 3.3 $\pm$ 0.8 months post initial transplant and achieved insulin independence for 1.0 $\pm$ 0.6 years (P=0.002). Descriptive baseline characteristics are presented in Table 2.1. Islet donor and graft characteristics are also described in Table 2.1. Recipient baseline characteristics were similar between both groups except for HbA1c and LI, which were higher in group 1CIT (HbA1c 9.2 $\pm$ 0.9% vs. 8.0 $\pm$ 0.9%, P=0.03 and LI 702 $\pm$ 274 vs. 359 $\pm$ 222, P=0.03) and BMI, which was lower in group 1CIT (23.9 $\pm$ 1.9 kg/m^2 vs. 27.0 $\pm$ 2.3 kg/m^2, P=0.01). There were no differences in donor characteristics between groups (Table 2.1). Although

there was no statistically significant difference in the number of islet equivalents infused at first transplant, because of their lower weight group 1CIT subjects received significantly higher islet equivalents per recipient body weight compared to group 2CIT subjects (9476 ± 4205 IEQ/kg vs. 5603 ± 846 IEQ/kg, P=0.03). However, once group 2CIT subjects received their second transplant they received a non-significantly higher amount of total islet equivalents per recipient body weight between groups (9476 ± 4205 IEQ/kg vs. 13094 ± 2711 IEQ/kg, P=0.07).

Islet Engraftment

The integrated assessment of islet engraftment using the BETA-2 score showed that in both groups evidence of engraftment was apparent at 1 week post-1CIT and appeared to continue over the next few weeks with BETA-2 score increasing and reaching a plateau by four to six weeks post-1CIT (Figure 2.1A). Group 1CIT subjects showed better early islet engraftment with significantly higher BETA-2 scores at 1 week compared to group 2CIT subjects (BETA-2 score 15 ± 3 vs. 9 ± 2, P=0.001). This difference was maintained until 16 weeks post-1CIT when most group 2CIT subjects had received their 2CIT (BETA-2 score 25 ± 4 vs. 17± 6, P=0.07).

Using fasting C-peptide to assess engraftment also showed evidence of islet engraftment with increasing level at 1 week post-1CIT. However, fasting C-peptide levels subsequently decreased and stabilized by approximately 4 weeks post-1CIT. Notably, there was generally no difference in fasting C-peptide between groups, including at 1 week post-1CIT (group 1CIT 1.08 ± 1.03 nmol/L vs. 2CIT 0.59 ± 0.24nmol/L, P=0.24) (Figure 2.1B).

The exception was at 6 weeks post-1CIT when C-peptide levels were higher in the group 1CIT (0.83 ± 0.24 nmol/L vs. 0.46 ± 0.18 nmol/L, P = 0.01). With islet transplantation, improvement in fasting blood glucose was observed at 1 week post-1CIT and remained stable thereafter in both groups (Figure 2.1C). Although group 1CIT subjects appeared to generally have lower fasting blood glucose levels compared to group 2CIT subjects, significant differences between both groups were not observed consistently. Insulin dose decreased with transplantation and similar to BETA-2 score was significantly different between groups, but from week 2 to 21 post initial CIT (P<0.05) (Supplemental Figure).

Glycemic Control

In keeping with improved fasting blood glucose, HbA1c was improved at 12 weeks post-1CIT in group 1CIT (9.2 ± 0.9% vs. 6.3 ± 0.7%, P<0.001) and group 2CIT (8.0 ± 0.9% vs. 6.4 ± 0.5%, P=0.002) (Figure 2.2). By 24 weeks, group 2CIT subjects had already received their 2CIT and both groups continued to have good glycemic control (group 1CIT 6.1 ± 0.4%, P<0.001 vs. baseline and group 2CIT 5.9 ± 0.5%, P<0.001, vs. baseline) (Figure 2.2). Notably, there was no difference in HbA1c between groups at 12 or 24 weeks post-1CIT (P=0.81 and P=0.59, respectively).

Early engraftment at one week predicts long term graft function

Two years post-1CIT, seven out of eight subjects in group 1CIT remained insulin independent compared to three out of seven subjects in group 2CIT (P=0.119). Long term graft function, as assessed by the BETA-2 score at 2 years, was higher in group 1CIT subjects compared to group 2CIT (22 ± 4 vs. 14 ± 7, P=0.02) (Figure 2.3) To explore the

relationship between early engraftment and long term graft function, linear regression analysis was performed. BETA-2 score at one week was associated with BETA-2 score at 3 and 24 months, whereas fasting blood glucose and fasting C-peptide at one week were not associated with graft function at any time point (Table 2.2).

CONCLUSIONS

Islet engraftment, as assessed by the BETA-2 score, takes place rapidly by the first week post-CIT and appears to be completed by 4-6 weeks. Considering the wide range of clinical outcomes post-CIT, we compared engraftment between subjects who had successful single islet transplantation to those who had required a second islet transplant in order to achieve insulin independence. Not surprisingly, subjects who did not have successful single islet transplant showed suboptimal islet engraftment with significantly lower BETA-2 score in the early period post-transplant. BETA-2 score achieved in this early period also appears to be important for long term function with BETA-2 score at 1 week being associated with BETA-2 score at 2 years post-CIT. Taken together, these data demonstrate that achieving optimal engraftment is important for successful single islet transplantation and that the BETA-2 score is a practical and effective tool for assessing islet engraftment.

Fasting C-peptide and fasting blood glucose also showed a pattern of rapid improvement in the first week post-CIT reflecting initial engraftment. However, unlike the BETA-2 score, they failed to consistently demonstrate differences in engraftment between group 1CIT and group 2CIT subjects. Thus, while fasting C-peptide and blood glucose levels may

provide information on initial islet engraftment, they are not ideal for subsequent assessment while insulin doses are being titrated post-CIT. Changes in insulin dose appear to drive increasing BETA-2 score and the significant difference in BETA-2 score between groups in the early period post-CIT. Therefore, changes in BETA-2 score reflect the function and time required for transplanted islets to provide clinically meaningful benefit. The contribution of A1C to changing BETA-2 score immediately post-CIT is limited by the fact that it is a chronic measure of glycemic control. However, as the BETA-2 score is also meant to assess long term graft function, inclusion of the A1C is relevant as it captures overall glycemic control, including potential contribution from post-prandial/random glucose levels.

Sub-optimal islet engraftment remains a major challenge in CIT with more than 50% of transplanted islets lost in the first few days post-transplantation (5, 9, 16). As a result, few patients are able to achieve insulin independence after a single islet transplant with most requiring at least two islet transplants for sustained clinical benefit (17). The ability to quantify islet engraftment provides an opportunity to identify variables that are associated with engraftment.

The superior engraftment observed in group 1CIT is especially striking given that the baseline HbA1c and LI were higher in this group. Interestingly, although pre-transplant PRA levels >15% have been associated with reduced islet graft survival (18), PRA levels were similar between groups in our current study and thus, do not appear to account for the observed differences in engraftment. In our study, subjects with optimal islet engraftment

and successful single CIT had lower BMI and received higher initial islet equivalents per body weight compared to those who had sub-optimal engraftment and required a second CIT. These data are in keeping with previous studies, which have demonstrated single islet transplant success in leaner patients and in patients who had received higher transplanted islet mass (19-21). However, we found that although the total islet equivalent dose received in both groups was similar once subjects in group 2CIT were re-transplanted, they still had inferior graft function at 2 years compared to group 1CIT subjects. Further studies assessing the BETA-2 score in larger and unselected cohorts of CIT patients are warranted to better characterize the relationship between islet equivalent dose and engraftment. It is possible that the higher weight and BMI in group 2CIT might have reflected a greater metabolic demand on the graft, although insulin dose per kg body weight were similar between groups at baseline. Total daily dose of insulin should perhaps be considered in islet transplant as a potential predictor of outcome since it reflects both body weight and insulin sensitivity and may better reflect metabolic demand.

Although the importance of primary graft function (using the original beta-score measured at one month after second or third CIT) to long term outcomes has been described (4), our data suggest that this may be dependent on very early engraftment with BETA-2 score as early as 1 week post-CIT being associated with long term graft function. Thus, it appears that having a favorable response to initial transplant, which may be due to as yet unmeasured donor or recipient factors, may be more important for long term graft function than the total amount of islets transplanted over time. Further studies are needed to identify

potential variables associated with islet engraftment in order that they may be targeted to optimize engraftment and improve CIT outcomes.

A potential limitation of the current analysis is exclusion of patients who received a second islet within 3 months of or more than 6 months from their first transplant. However, this was necessary in order to assess early engraftment independent of the effects of a second transplant, while also capturing the effect of re-transplantation during study follow up. The study period of 2009 and 2014 limited the number of subjects included in the current analysis. However, this allowed for comparison of engraftment between groups without the confounding effects of changing CIT protocols over time; notably, subjects in both group 1CIT and 2CIT received similar immunosuppressive protocols and did not have difference in average tacrolimus levels. As it remains difficult to directly assess beta cell mass *in vivo,* we were unable to distinguish between the contribution of beta cell mass compared to beta cell secretory function when measuring the BETA-2 score. As imaging technology advances, studies characterizing the relationship between beta cell mass and the BETA-2 score will be important in furthering our understanding of islet engraftment.

Islet engraftment has previously been difficult to assess and as a result little is known about it in the clinical setting. Taking advantage of the BETA-2 score, we characterized islet engraftment and function in the early period post CIT and show that engraftment occurs rapidly in the first few weeks following islet transplantation and that this early period is critical for long term graft function. We also demonstrate that the BETA-2 score is an effective tool for monitoring islet function on a frequent and regular basis. Thus, the

BETA-2 score is a practical measure of islet function that can be used clinically to monitor patients, as well as in the development and evaluation of interventions targeted at improving islet engraftment and ultimately CIT outcomes.

ACKNOWLEDGEMENTS

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TABLES

Table 2.1: Recipient, donor and graft characteristics

	All patients (n = 15)	Group 1CIT (n = 8)	Group 2CIT (n = 7)	P
Recipient Characteristics				
Gender (male/female)(%)	5 (33)/10 (67)	2 (25)/6 (75)	3 (43)/4 (57)	0.61
Age (years)	55.6 ± 9.9	56.8 ± 9.4	54.3 ± 11.1	0.64
Diabetes duration (years)	34.9 ± 13.6	32.8 ± 13.4	37.3 ± 14.4	0.54
Weight (kg)	68.5 ± 10.8	64.1 ± 8.1	73.4 ± 11.9	0.10
BMI (kg/m^2)	25.4 ± 2.6	23.9 ± 1.9	27.0 ± 2.3	0.01
HbA1c (%)	8.6 ± 1.1	9.2 ± 0.9	8.0 ± 0.9	0.03
Fasting blood glucose (mmol/L)	12.3 ± 5.4	13.5 ± 4.9	11.0 ± 6.2	0.41
Lability index	542 ± 300	702 ± 274	359 ± 222	0.03
HYPO score	1584 ± 1676	1894 ± 2271	1230 ± 513	0.20
Basal insulin dose (units/kg per day)	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.96
PRA class I and II				
Either of one < 15%	12 (80)	5 (62)	7 (100)	0.20
Either of one >15%	3 (20)	3 (38)	0 (0)	
Immunosuppression (alemtuzumab/thymoglobulin)	14 (93)/1 (7)	7 (88)/1 (12)	7 (100)/0 (0)	1.00
Trough tacrolimus level (ug/L)*	8.9 ± 1.4	9.2 ± 2	8.7 ± 0.4	0.53
Donor and Graft Characteristics				
Age	53.1 ± 14.3	50.4 ± 12.9	56.1 ± 16.3	0.46
BMI	33.3 ± 8.5	37.2 ± 9.6	28.8 ± 4.3	0.05
Cold ischemic time	9.2 ± 2.8	9.2 ± 3.0	9.3 ± 2.9	0.98
Islet viability	84.0 ± 5.5	85.6 ± 4.5	82.3 ± 6.3	0.26
Islet purity	61.2 ± 15.8	64.5 ± 13.2	57.3 ± 18.6	0.40
Stimulation index	2.8 ± 1.4	3.3 ± 2.1	2.4 ± 0.6	0.34
1CIT IEQ	525364 ± 274102	624189 ± 348429	412422 ± 75944	0.14
1CIT IEQ/kg	7669 ± 3626	9476 ± 4205	5603 ± 846	0.03
2CIT IEQ			519886 ± 176138	
2CIT IEQ/kg			7491 ± 2312	
Total IEQ	767978 ± 328107	624189 ± 348429	932308 ± 224685	0.07
Total IEQ/kg	11164 ± 3935	9476 ± 4205	13094 ± 2711	0.07

Group 1CIT subjects became insulin independent after a single CIT. Group 2CIT subjects required a second CIT before achieving insulin independence. Data are expressed as mean ± SD and n (%). CIT, clinical islet transplant; 1CIT, first CIT; 2CIT, second CIT; PRA, panel reactive antibodies. IEQ, islet equivalents. * Trough tacrolimus levels represent the average of values to 1 month after 1CIT. 1CIT and 2CIT were compared by two-tailed t-test or Fisher's exact test as appropriate.

Table 2.2: Univariate analysis of BETA-2 Score at 3 and 24 months post initial CIT with measures of engraftment at 1 week post initial CIT

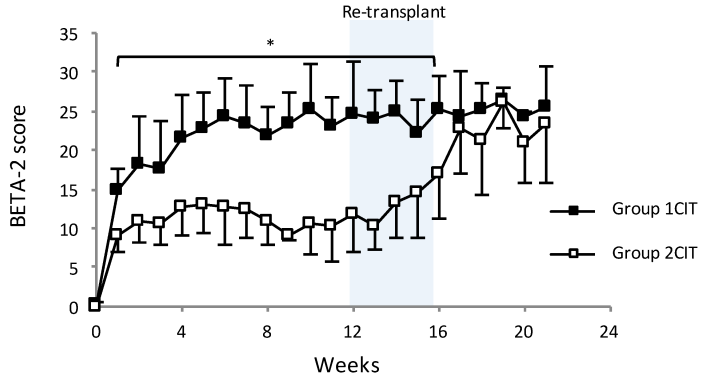
	BETA-2 at 3 months			BETA-2 at 24 months		
	β	P-value	R^2	β	P-value	R^2
BETA-2 at 1 week	1.753	0.002	0.635	0.977	0.034	0.496
FBG at 1 week	0.946	0.771	0.013	0.522	0.803	0.008
FCP at 1 week	-1.008	0.409	0.099	-0.638	0.494	0.069

CIT, clinical islet transplant; FBG, fasting blood glucose; FCP, fasting C-peptide.

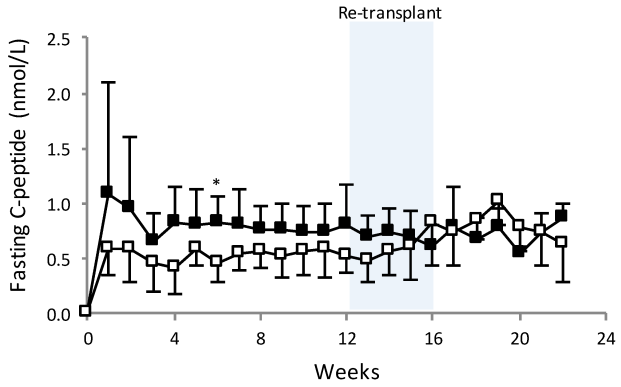
FIGURES

Figure 2.1: BETA-2 score, fasting C-peptide, and fasting blood glucose post clinical islet transplantation (CIT)

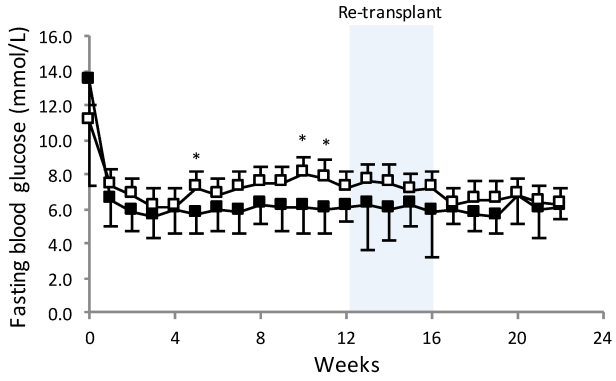
A.



B.

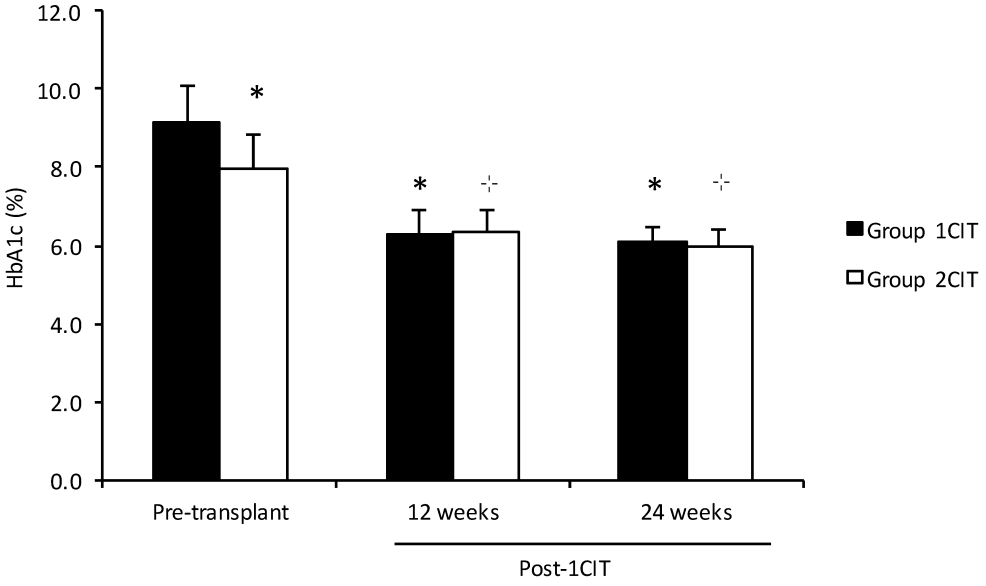


C.



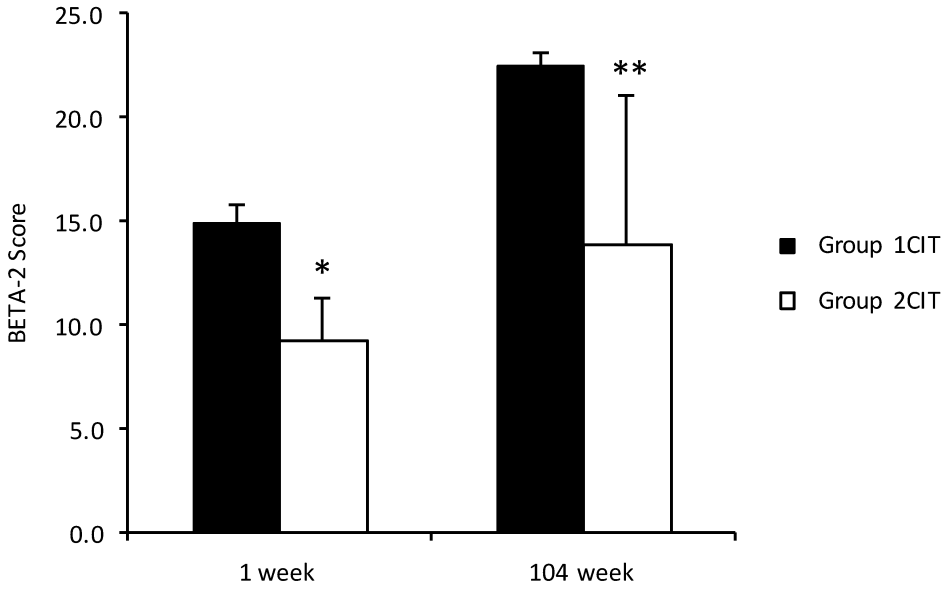
BETA-2 score (A), fasting C-peptide (B) and fasting blood glucose (C) in the first 6 months post initial clinical islet transplant (CIT). Group 1CIT subjects became insulin independent after a single CIT (closed squares). Group 2CIT subjects required a second CIT before achieving insulin independence (open squares). Shaded area indicates when group 2CIT received their second CIT. *P<0.05, group 1CIT vs. group 2CIT.

Figure 2.2: HbA1c pre and post initial clinical islet transplantation (CIT)



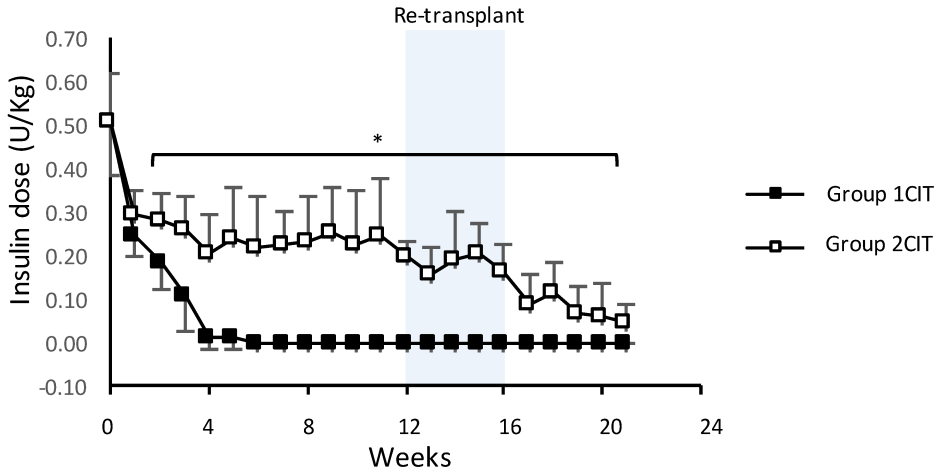
Group 1CIT patients became insulin independent after a single CIT. Group 2CIT patients required a second CIT before achieving insulin independence. * P<0.05 vs. group 1CIT pre-transplant; ⊹ P<0.05 vs. group 2CIT pre-transplant.

Figure 2.3: BETA-2 score at 1 and 104 weeks post initial clinical islet transplantation (CIT)



Group 1CIT patients became insulin independent after a single CIT. Group 2CIT patients required a second CIT before achieving insulin independence. * P<0.05, vs. group 1CIT at 1 week; ** P<0.05, vs. group 1CIT at 104 weeks.

Figure 2.4 (Supplemental Figure) Insulin dose post clinical islet transplantation (CIT)



Insulin dose in the first 6 months post initial clinical islet transplant (CIT). Group 1CIT subjects became insulin independent after a single CIT (closed squares). Group 2CIT subjects required a second CIT before achieving insulin independence (open squares). Shaded area indicates when group 2CIT received their second CIT. *P<0.05, group 1CIT vs. group 2CIT.

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Chapter 3 : BETA-2 score as a Clinical Outcome in Type 1 Diabetes Intervention Trials

INTRODUCTION

Outcome assessments in clinical trials must address important aspects of disease management. In type 1 diabetes (T1D), this includes the preservation of beta cell function and maintenance of glucose homeostasis. Stimulated C-peptide is generally accepted as the most suitable primary outcome in T1D intervention trials (1). However, while this provides sensitive and standardized information on beta cell function, it does not provide information on clinical outcomes.

Measures of glycemic control and insulin usage are often used as secondary outcomes. However, certain limitations should be noted. For example, hemoglobin A1C (A1C) provides a measure of overall glycemic control, but fails to capture day-to-day variation in glucose levels. Furthermore, differences in A1C are not expected between treatment and control groups, given that both groups should share the same A1C target as part of standard care. Fasting blood glucose is an alternative measure of glycemic control, but is highly variable being affected by beta cell function, insulin dose, diet, etc. Insulin use is another outcome of interest; insulin independence has the advantage of being a patient important outcome that is also easily interpretable. Practically, however, insulin independence is a stringent outcome which is difficult to achieve, and some statistical power is lost when converting insulin dose from a continuous variable to a dichotomous one (2).

Defining appropriate clinical outcomes is similarly challenging in islet transplantation, where the restoration of beta cell function results in a wide spectrum of outcomes (3-6). In this field, stimulated C-peptide has been considered the gold standard for measuring beta cell function. However, measuring stimulated C-peptide by mixed meal tolerance test is time- and labor-intensive (7) and therefore not practical for the routine follow up of islet transplant patients. To that end, the BETA-2 score was developed as an alternative index of beta cell function that also serves as an overall clinical assessment. The score is conveniently calculated from a single blood sample and includes fasting C-peptide, A1C, fasting blood glucose and insulin dose (8). It is also easily interpretable with a score of >20 excluding any degree of mild glucose intolerance and score $\geq$ 15 detecting insulin independence in islet transplant patients (8).

The aim of this study was to evaluate the BETA-2 score as an outcome measure in T1D intervention trials. We assessed the BETA-2 score in two previously completed pilot randomized controlled clinical trials including the ATG/G-CSF study, which evaluated anti-thymocyte globulin (ATG) in combination with granulocyte colony stimulating factor (G-CSF) (9) and the exercise to preserve beta cell function in recent onset type 1 diabetes mellitus (EXTOD) trial, which evaluated exercise as an intervention to preserve beta cell function in T1D patients (10).

METHODS

Study populations

Data evaluated in this study came from two randomized, placebo controlled trials in T1D patients. Details regarding the study design, patient population and intervention of each study have previously been reported (9, 10). Each study is briefly detailed below.

ATG/G-CSF was a singled blinded study that evaluated the efficacy of low dose ATG and G-CSF in patients with established T1D (duration >4 months and <2 years). 25 subjects were randomized 2:1 to receive ATG (2.5 mg/kg IV) followed by pegylated G-CSF (6 mg subcutaneously every 2 weeks for 6 doses) (n = 17) or placebo (n = 8). The primary outcome was 1 year change in area under the curve (AUC) C-peptide following 2-hour MMTT.

The EXTOD trial was an unblinded pilot study that randomized newly diagnosed T1D patients (duration <12 weeks) 1:1 to exercise intervention (maintenance of baseline exercise intensity for a minimum of 150 minutes per week with the aim of increasing to 240 minutes per week of vigorous exercise) (n = 30) or usual care for 1 year (n = 28). The study was designed to assess barriers to exercise programs with the primary outcome of interests including recruitment and adherence rates. The secondary outcome was 1 year AUC C-peptide following 2-hour MMTT. Retrospective analysis of EXTOD trial data revealed 3 patients in the treated group and 11 patients in the control group that were negative for T1D associated autoantibodies including glutamic acid decarboxylase (GAD),

insulinomas-associated-2 (IA-2) and zinc transporter 9 (ZnT8)). These patients were assumed to have type 2 diabetes and therefore excluded from the current analysis.

Assessment

The BETA-2 score was calculated at baseline, 6 and 12 months. Individual components of the BETA-2 score, including fasting C-peptide, fasting blood glucose, insulin dose and A1C were also assessed.

$$\text{BETA-2 Score} = \frac{\sqrt{(\text{fasting C-peptide}) \times (1 - \text{insulin dose})}}{\text{fasting plasma glucose} \times \text{HbA1c}} \times 1000 \quad (8)$$

Adjusted change in BETA-2 score from baseline was calculated by dividing the change in BETA-2 score (baseline to 3 and 6 months, respectively), by baseline BETA-2 score.

Statistics

Statistical analyses were performed using Stata version 14.1 (StataCorp, College Station, TX). Missing data were handled by pairwise deletion method. Descriptive statistics were expressed as mean $\pm$ standard deviation (SD). Two-tailed t-test and Fisher's exact test were used to compare groups as appropriate. Linear regression was used to evaluate the relationship between AUC C-peptide and BETA-2 score and the relationship between AUC C-peptide and adjusted change in BETA-2.

RESULTS

Baseline characteristics for each study are provided in Table 3.1. There were no significant differences between the control and intervention group in the ATG/G-CSF trial (9). However, in the EXTOD trial there were differences between the groups, with a greater proportion of males (0.82 vs.0.48, P=0.03), younger age at diagnosis of T1D (25.9 $\pm$ 6.3 years vs. 34.0 $\pm$ 11.8 years, P=0.01) and lower GAD antibody titers (289.30 $\pm$ 317.93 vs. 490.68 $\pm$ 299.18, P = 0.04) in the control group compared to the intervention group. Importantly, in both trials, AUC C-peptide levels were similar between intervention groups at baseline (ATG/G-CSF control 0.71 $\pm$ 0.64 nmol/l/min vs. treated 0.71 $\pm$ 0.48 nmol/l/ml, and EXTOD control 0.92 $\pm$ 0.40 nmol/l/min vs. treated 0.94 $\pm$ 0.39 nmol/l/min). There were also no differences in BETA-2 scores at baseline (ATG/G-CSF control 12.0 $\pm$ 10.6 vs. treated 9.2 $\pm$ 5.3, and EXTOD control 7.3 $\pm$ 4.2 vs. treated 7.7 $\pm$ 3.4).

As previously described (9), there was a tendency towards improved beta cell function in the ATG/G-CSF treated group. At 1 year, AUC C-peptide was 0.74 $\pm$ 0.47 nmol/l/min in treated subjects compared to 0.43 $\pm$ 0.32 nmol/l/min in control subjects (P=0.05). However, glycemic control at 1 year was not different between groups (A1C control 6.71 $\pm$ 0.60% vs. treated 7.34 $\pm$ 2.15%, P = 0.47; and fasting blood glucose control 7.5 $\pm$ 2.2 mmol/L vs. treated 7.1 $\pm$ 1.2 mmol/L, P = 0.60) (Figure 3.1). Similarly, insulin dose was not different between groups (control 0.54 $\pm$ 0.29 unit/kg vs. treated 0.48 $\pm$ 0.40 unit/kg, P=0.69). BETA-2 score, though numerically higher in treated subjects, was not statistically different between groups at 1 year (control 5.2 $\pm$ 5.4 vs. treated 9.73 $\pm$ 5.4, P=0.11). However, BETA-2 score showed a trend that was similar to AUC C-peptide, with ongoing

decline of beta cell function in control subjects compared to apparent maintenance of function in treated subjects (Figure 3.1).

In the EXTOD trial, exercise did not improve beta cell function (1 year AUC C-peptide control 0.69 $\pm$ 0.44 vs. treated 0.61 $\pm$ 0.30 nmol/l/min, P = 0.51) (Figure 3.2). Similarly, there were no differences in BETA-2 score (control 7.8 $\pm$ 4.8 vs. treated 7.1 $\pm$ 4.1, P= 0.69), insulin dose (control 0.31 $\pm$ 0.22 unit/kg vs. 0.25 $\pm$ 0.12 unit/kg, P = 0.34) or measures of glycemic control (A1C control 7.56 $\pm$ 1.61% vs treated 7.57 $\pm$ 1.11%, P= 0.9 and fasting blood glucose control 8.2 $\pm$ 2.7 mmol/l vs. treated 8.3 $\pm$ 2.8, P=0.93) between groups (Figure 3.2).

Figure 3.3 shows scatter plots of AUC C-peptide and BETA-2 score. BETA-2 score was significantly correlated with AUC C-peptide at 0, 6 and 12 months of treatment in both trials. The correlations were strong in the ATG/G-CSF trial with r values 0.685 – 0.853 (P= 0.000 – 0.002) compared to the EXTOD trial where correlations were weaker with r values 0.391– 0.657 (P= 0.000 – 0.010). Relationship between BETA-2 score measured at 3 and 6 months with AUC C-peptide measured later at 1 year was explored using the ATG/G-CSF trial data. Both BETA-2 score at 3 months (r = 0.60, P = 0.22) and 6 months (r = 0.80, P = 0.000) correlated with 1 year AUC C-peptide (Table 3.2). Adjusted change in BETA-2 score from baseline to 6 months was significantly correlated with AUC-C-peptide at 1 year (r = 0.68, P = 0.01), whereas there was no correlation between adjusted change in BETA- 2 score from baseline to 3 months and 1 year AUC C-peptide (r = 0.10, P = 0.77).

DISCUSSION

Assessing beta cell function and defining clinically relevant outcomes remain challenges in T1D intervention trials (11). Measurement of C-peptide alone does not provide clinical information that is relevant to patients and clinicians alike. Furthermore, measures of glycemic control or insulin use are inadequate as they are inter-dependent and therefore insufficient when considered individually. As such, having a single measure which incorporates information on beta cell function and metabolic outcome is desirable. In the current study, we assessed the BETA-2 score, which was originally developed in clinical islet transplantation (8) as a potential outcome in two previously completed T1D intervention trials.

In the ATG/G-CSF study, treated subjects appeared to have maintained beta cell function and yet, fasting blood glucose, A1C and insulin were not different between treated and control groups. Conversely, the BETA-2 score, which is a composite of these measures, showed a similar pattern to AUC C-peptide with appreciable, although not statistically significant, separation between control and treated groups. In the EXTOD trial, there were no differences in fasting glucose, A1C, insulin dose or BETA-2 score between groups. However, this was expected as exercise failed to improve beta cell function in this study. We examined the relationship between the BETA-2 score and AUC C-peptide in both studies and found that they were well correlated at 0, 6 and 12 months. Of note, correlation between AUC C-peptide and BETA-2 score was higher in the ATG/GCSF trial compared to the EXTOD trial. This likely reflects differences between trial subjects; the ATG/G-CSF trial subjects had longer duration of T1D and therefore lower AUC C-peptide, but good

glycemic control compared to EXTOD trial subjects who were newly diagnosed with T1D and had higher AUC C-peptide, but sub-optimal glycemic control. Contrary to what appears to be better beta cell function based on stimulated C-peptide in EXTOD trial subjects, the lower BETA-2 score captures the fact that this level of beta cell function is inadequate for the level hyperglycemia observed and likely also reflects a degree of beta cell dysfunction as a result of glucotoxicity. The BETA-2 score measured at 3 and 6 months correlated with AUC C-peptide measured at 1 year in the ATG/G-CSF trial. Taken together, the BETA-2 score appears to be useful as an overall assessment of metabolic status and given its relationship with AUC C-peptide, it may prove to be a clinically meaningful surrogate of beta cell function.

Surrogate endpoints must fulfill at least two criteria: 1) correlate with the true endpoint, and 2) be as responsive to treatment as the true endpoint (12, 13). Although we show the former here, further studies are needed to establish the latter. Our ability to assess the responsiveness of the BETA-2 score was limited by the inclusion of trials which showed weak to no treatment effect. Additionally, the BETA-2 score was not a pre-specified outcome in either trial, and as a result missing data decreased statistical power to detect differences in the BETA-2. With respect to the EXTOD trial, it important to note that the erroneous inclusion of type 2 diabetic patients and subsequent removal of these patients from the current analysis with resulting differences in baseline characteristics between groups makes interpretation of this data difficult.

In the next phase of work, we propose a meta-analysis of treatment effects on the BETA-2 score in trials which showed significant beta cell preservation (13-15). Although this may be challenging given that few T1D intervention trials have met their primary endpoint, there are some which have, and would be worth analyzing (16-19). Studies determining whether the BETA-2 score is useful in the early detection of responders and non-responders are also warranted. To further validate the BETA-2 score, it will also be important to understand how it changes with disease progression and whether it differs within the T1D population which is increasingly being recognized as a heterogeneous population. To this end, we propose to analyze existing TrialNet data which includes completed and ongoing mechanistic and natural history studies in T1D patients (20). Other considerations for future studies include comparing the performance of the BETA-2 score to other surrogates of beta cell function (i.e. SUITO, CP/G, and HOMA2-B) (21-23) and validation of the BETA-2 score in the presence of insulin resistance.

Clinical islet transplantation and intervention trials in T1D both center on the use of immune modulating therapies to prevent the immune destruction of pancreatic beta cells. Treatments in either domain must principally preserve beta cell function and improve glucose homeostasis in order to be considered effective. We have shown here that the BETA-2 score is a simple tool which provides relevant clinical information and correlates well with AUC C-peptide. Further studies are warranted to determine the responsiveness and reliability of the BETA-2 score as a surrogate endpoint in T1D intervention trials.

TABLES

Table 3.1: Baseline characteristics

	ATG/G-CSF Trial		EXTOD Trial	
	Intervention	**Control**	**Intervention**	**Control**
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Sex (M, male; F, Female)	12 M/5 F	5 M/3 F	13 M/14 F	14 M/3 F*
Age at diagnosis (years)	23.6 (10.0)	23.6 (10.6)	34.0 (11.8)	25.9 (6.3)*
Time from diagnosis (years)	1.0 (0.6)	0.9 (0.5)	< 12 weeks	
BMI (kg/m^2)	25.4 (5.2)		24.9 (3.4)	23.8 (2.2)
AUC C-peptide 2 hour MMT (nmol/l/min)	0.71 (0.48)	0.71 (0.64)	0.94 (0.39)	0.92 (0.40)
BETA-2 score	9.2 (5.3)	12.0 (10.6)	7.7 (3.4)	7.4 (4.2)
A1C (%)	6.69 (1.09)	6.03 (0.97)	8.9 (2.2)	9.1 (2.1)
Daily insulin usage (units/kg per day)	0.44 (0.49)	0.45 (0.32)	0.27 (0.20)	0.34 (0.17)
Fasting blood glucose (mmol/l)	6.3 (1.2)	6.2 (1.9)	7.6 (3.0)	7.4 (2.0)
Fasting C-peptide (nmol/l)	0.33 (0.29)	0.42 (0.25)	0.38 (0.16)	0.39 (0.17)
GAD autoantibodies (units/ml)	292.12 (307.1)	357.13 (312.4)	490.68 (299.18)	289.30 (317.93)*
ZnT8 autoantibodies (units/ml)	0.16 (0.32)	0.25 (0.31)	11.82 (19.18)	41.15 (60.96)*
IA-2 autoantibodies (units/ml)	75.18 (125.0)	158.0 (162.7)	101.40 (128.2)	167.32 (196.09)

M, male; F, female. *P<0.05 vs. intervention.

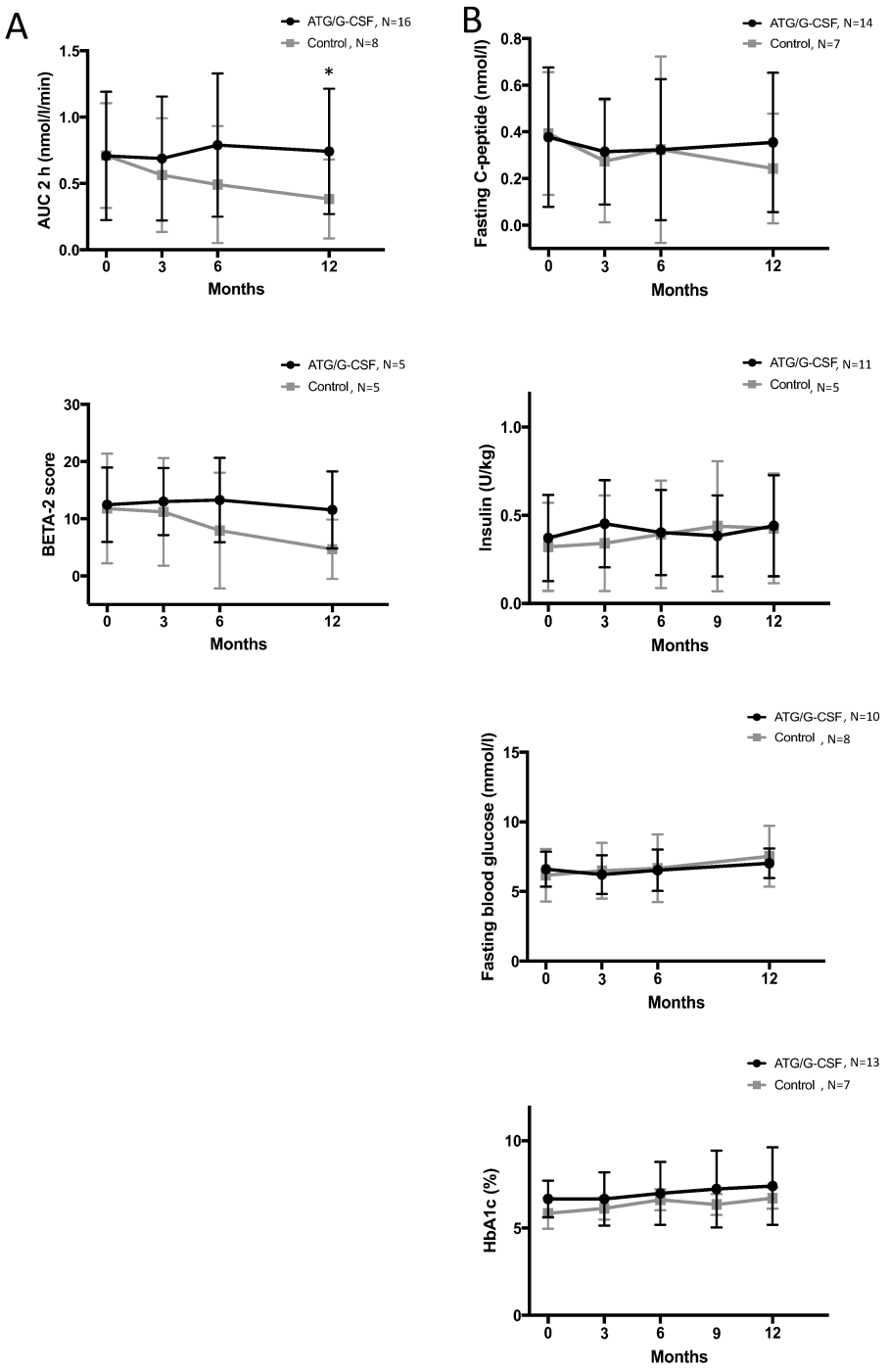
Table 3.2: Correlation between AUC C-peptide at 12 months with BETA-2 score

	n	R	P
BETA-2 Score at 3 months	14	0.60	0.022
BETA-2 Score at 6 months	15	0.80	0.000
Adjusted change in BETA-2 at 3 months*	12	0.10	0.767
Adjusted change in BETA-2 score at 6 months*	13	0.68	0.011

Correlation between AUC C-peptide at 12 months with BETA-2 score (at 3 and 6 months) and change in BETA-2 score (from baseline to 3 months and baseline to 6 months) based on data from the ATG/G-CSF trial. *Change from baseline adjusted for baseline BETA-2 score.

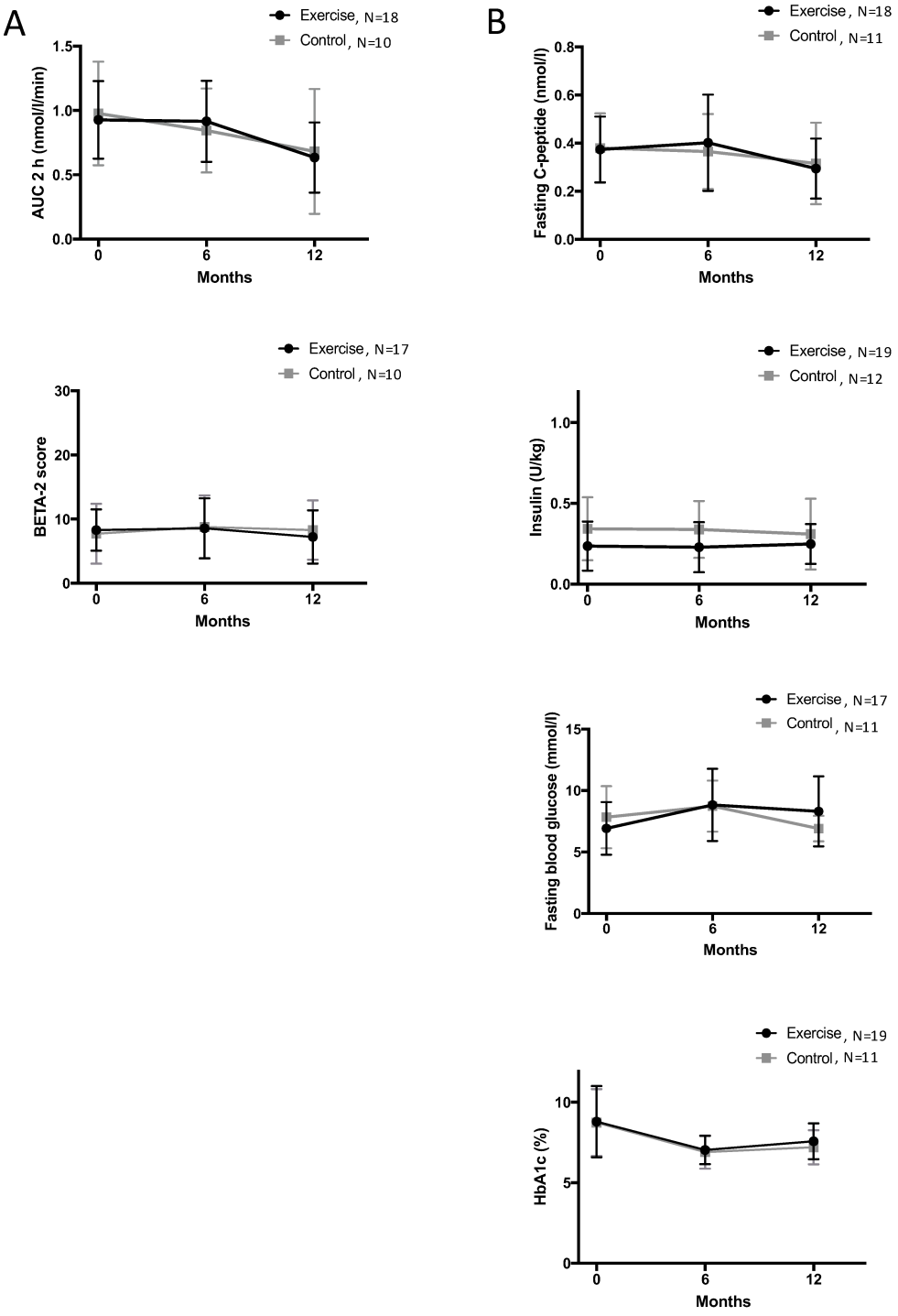
FIGURES

Figure 3.1: Beta cell function and metabolic control in patients from the ATG/G-CSF trial



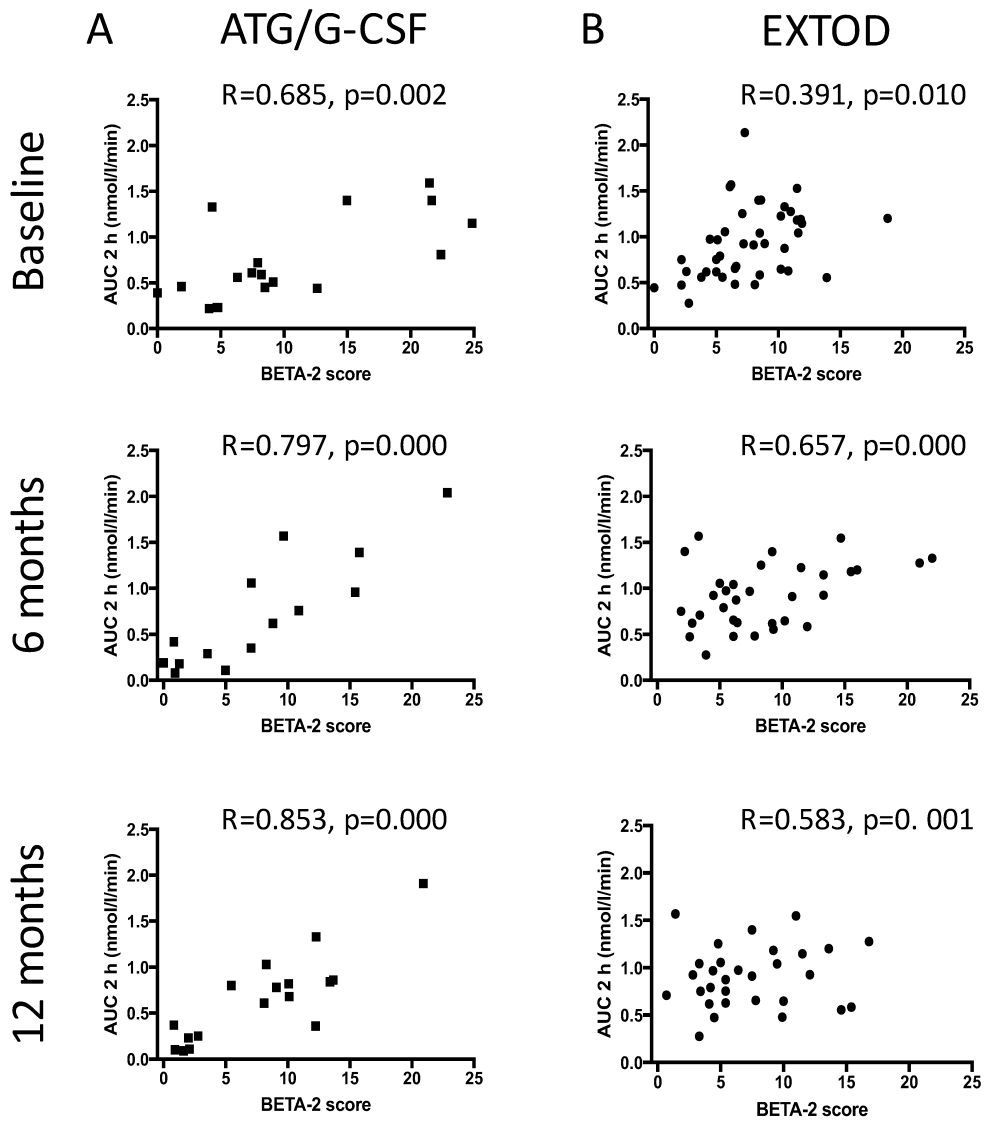
Beta cell function (A) and metabolic control (B) in patients from the ATG/G-CSF trial. AUC C-peptide are shown as total AUC divided by 120 minutes. Data are presented as mean ± SD. *P=0.05.

Figure 3.2: BETA-2 score and metabolic control in patients from the EXTOD



Beta cell function (A) and metabolic control (B) in patients from the EXTOD trial. AUC C-peptide are shown as total AUC divided by 120 minutes. Data are presented as mean ± SD. *P=0.05.

Figure 3.3: Correlation between AUC C-peptide and BETA-2 score



Correlation between AUC C-peptide and BETA-2 score at baseline, 6 months and 12 months of treatment. AUC C-peptide are shown as total AUC divided by 120 minutes.

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Chapter 4 : Conclusion

Defining clinically relevant outcomes is a challenge in both type 1 diabetes (T1D) intervention trials and clinical islet transplantation (CIT). In CIT, the need for the frequent and routine monitoring of beta cell function makes MMTTs impractical, especially during islet engraftment which occurs over the first days to weeks post-transplant. In T1D intervention trials, stimulated C-peptide alone is inadequate in assessing whether a potential intervention has a clinically important effect. Therefore, having a simple index of beta cell function and metabolic status, such as the BETA-2 score, is desirable in both CIT and T1D trials. In this study, I showed that the BETA-2 score is a practical and effective tool for assessing islet engraftment in CIT patients. I also showed that the BETA-2 score can be applied to T1D intervention trials and that it correlates well with stimulated C-peptide.

Using the BETA-2 score, I described for the first time the time course for islet engraftment in CIT. We also showed that optimal engraftment is important for successful single islet transplantation. Our study was limited by strict inclusion criteria and retrospective design. Larger studies that include patients without consideration for timing of subsequent transplant or post-transplant outcomes (i.e. insulin independence) are needed to further characterize islet engraftment in CIT. Importantly, our study does serve as proof of concept that the BETA-2 score can be used routinely post-CIT and that it may be useful in distinguishing patients with optimal versus sub-optimal graft function. Thus, the BETA-2 score is likely useful in prospective studies assessing new transplant techniques or

immunosuppressive strategies in CIT. Longitudinal studies of BETA-2 score will also allow for correlation between the BETA-2 score and clinical endpoints which are more difficult to capture in trial settings including hypoglycemia and diabetes associated micro- and macro-vascular complications. Other potential application of the BETA-2 score includes its use in the early detection of graft failure allowing for earlier intervention and/or repeat transplantation.

In the latter part of this study, the BETA-2 score was retrospectively calculated in the ATG/G-CSF trial and in the EXTOD trial. We showed that the BETA-2 score was significantly associated with stimulated C-peptide in both studies. However, despite the modest treatment effect observed in the ATG/G-CSF trial, we found no difference in the BETA-2 score between treated and control subjects. This may have been related to the fact that the treatment effect was only modest. Missing data and decreased statistical power may have also been contributing factors. Nonetheless, the observed relationship between the BETA-2 score and stimulated C-peptide is encouraging and warrants further study to evaluate the responsiveness of the BETA-2 score as a clinical endpoint.

In conclusion, we have shown that the BETA-2 score is a practical index of beta cell function and metabolic status. It allows early and frequent monitoring of response to CIT and correlates well with stimulated C-peptide in T1D intervention trials. As such, it is likely useful in the clinical setting for monitoring of beta cell function and also in clinical trials for evaluating treatments designed to preserve beta cells.

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