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Uitbeijerse, B.S.; Nijhoff, M.F.; Sont, J.K.; Koning, E.J.P. de

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# Fasting parameters for estimation of stimulated $\beta$ cell function in islet transplant recipients with or without basal insulin treatment

Bas S. Uitbeijerse<sup>1</sup>  | Michiel F. Nijhoff<sup>1</sup>  | Jacob K. Sont<sup>2</sup>  | Eelco J. P. de Koning<sup>1</sup> 

<sup>1</sup>Department of Internal Medicine, Leiden University Medical Center, Leiden, the Netherlands

<sup>2</sup>Department of Biomedical Data Sciences, Section Medical Decision Making, Leiden University Medical Center, Leiden, the Netherlands

## Correspondence

Eelco J. P. de Koning  
Email: e.j.p.de\_koning@lumc.nl

In order to assess  $\beta$  cell secretory capacity after islet transplantation, standardized mixed meal stimulation tests are often used. But these tests are cumbersome and the effect of exogenous insulin on the test results is unclear. The aim of our study was to determine to what extent fasting glycemic indices can estimate stimulated  $\beta$  cell function in islet transplant recipients with and without basal insulin. In total 100 mixed meal stimulation tests, including 31 with concurrent basal insulin treatment, were performed in 36 islet transplant recipients. In a multivariate model, fasting C-peptide and fasting glucose together estimated peak C-peptide with  $R^2 = .87$  and area under the curve (AUC) C-peptide with a  $R^2 = .93$ . There was a larger increase of glucose during tests in which exogenous insulin was used (+7.9 vs +5.3 mmol/L,  $P < .001$ ) and exogenous insulin use was associated with a slightly lower estimated peak C-peptide (relative change:  $-15\%$ ,  $P = .02$ ). In islet transplant recipients the combination of fasting C-peptide and glucose can be used to accurately estimate stimulated  $\beta$  cell function after a mixed meal stimulation test, whether exogenous basal insulin is present or not. These data indicate that graft function can be reliably determined during exogenous insulin treatment and that regular islet graft stimulation tests can be minimized.

## KEYWORDS

clinical research/practice, diabetes, endocrinology/diabetology, insulin/C-peptide, islet transplantation, islets of Langerhans

## 1 | INTRODUCTION

Pancreatic islet transplantation is, together with whole pancreas transplantation, the only treatment that potentially obviates the need for insulin treatment in patients with type 1 diabetes mellitus and other forms

of severe  $\beta$  cell deficiency.<sup>1</sup>  $\beta$  cell function posttransplant is an important indicator of the success of  $\beta$  cell replacement therapy and is used in the Igls criteria for assessment of  $\beta$  cell replacement therapy outcome.<sup>2</sup>

$\beta$  cell function is preferably measured by C-peptide, as endogenous insulin is partially cleared by the liver and suffers from cross-reactivity

**Abbreviations:** AIC, Akaike information criterion; AUC, area under the curve; BI, basal insulin; BIC, Bayesian information criterion; BMI, body mass index; CI, confidence interval; CP/G, C-peptide/glucose ratio; CP/GCR, C-peptide/glucose creatinine ratio; CSII, continuous subcutaneous insulin infusion; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; IAK, islet after kidney transplantation; IAL, islet after lung transplantation; IEQ, islet equivalent; IQR, interquartile range; ITA, islet transplantation alone; MMTT, mixed meal tolerance test; NI-IID, no insulin, insulin independent; NI-ITS, no insulin, insulin temporarily stopped; SD, standard deviation; SUITO, secretory units of islet transplant objects; TEF, transplant estimated function; TP-IAT, total pancreatectomy with islet autotransplantation.

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with exogenous insulin in many assays. Because secretion by the  $\beta$  cell is dynamically regulated, with an important role for carbohydrates in food and meal-related incretin hormones as triggers of insulin secretion, the glucose concentration and prandial state (fasting/nonfasting) are very important to accurately interpret C-peptide measurements. To standardize the assessment of  $\beta$  cell secretory capacity,  $\beta$  cell stimulation tests such as the intravenous glucagon, oral glucose, intravenous arginine, intravenous glucose, or mixed meal tests are preferred.<sup>3-5</sup> Although beta-cell secretory capacity derived from glucose-potential of arginine-induced insulin secretion provides the best estimate of functional  $\beta$  cell mass,<sup>5</sup> being relatively independent of recipient-related factors, the mixed meal stimulation test (also termed mixed meal tolerance test [MMTT]) is the gold standard for measuring  $\beta$  cell function during clinical follow-up because it is the most physiologic stimulus.<sup>6</sup> However, the MMTT and the other stimulation tests are cumbersome and time consuming, leading to high costs and infrequent graft function assessment. There is also the question whether these tests are still useful when a patient is on concurrent insulin therapy, further limiting assessment of  $\beta$  cell secretory capacity. For this reason, we investigated whether a set of fasting indices could estimate stimulated  $\beta$  cell function after a MMTT in patients with and without concurrent exogenous insulin use. Such indices could lead to lower costs, more frequent measurements, and thus better understanding of the course of graft function after islet transplantation and possibly even earlier detection of graft rejection.

## 2 | MATERIAL AND METHODS

The study was performed in accordance with the ethical principles of the Helsinki declaration and relevant laws within the Netherlands ("General Data Protection Regulation" and "Medical Treatment Agreement Act [WGBO]"). The ethics committee of the Leiden University Medical Center provided a statement that no ethical permission was needed for this study (G18.075).

### 2.1 | Patients

This was a retrospective study of consecutive patients who underwent at least one eligible MMTT between January 1, 2010 and February 13, 2019 after an islet transplantation and who had no allogeneic pancreas graft in situ. They included both islet allotransplantation and islet autotransplantation recipients at the Leiden University Medical Centre. The islet isolation and transplantation were performed according to previously published protocols.<sup>1,7-9</sup> The decision to continue, discontinue, or reinitiate insulin treatment after islet transplantation was made on clinical grounds.

### 2.2 | Mixed meal stimulation tests

The MMTT is a standard procedure in our center to assess islet graft function at 3 months, 1 year, and from then yearly after islet

transplantation. However, if total graft failure is established (undetectable C-peptide [ $<0.03$  nmol/L] during the MMTT), no more MMTTs are performed. Patients were instructed to arrive at the outpatient clinic in the morning while fasting for at least 8 hours. When patients used long-acting insulin, they were instructed to withhold the long-acting insulin the previous day when this was considered feasible (aiming at a fasting glucose 4.0-10.0 mmol/L). When patients used continuous subcutaneous insulin infusion (CSII), they were instructed to stop the insulin pump at midnight. If stopping CSII at midnight or withholding long-acting insulin injection during the previous day was not considered feasible, the dose of long-acting insulin or basal rate of the CSII was adjusted to prevent hypoglycemia before the start of the MMTT. Furthermore, no bolus insulin was allowed within 4 hours of the MMTT. After a vein was cannulated, the patients were instructed to ingest 360 mL BOOST<sup>®</sup> (50 g of carbohydrates, 22.7 g of protein, and 9 g of fat) until November 1, 2015. As BOOST<sup>®</sup> was no longer available in the Netherlands from November 2015, a switch of the mixed meal was necessary. This mixed meal consisted of 270 mL Nutridrink<sup>®</sup> (49.7 g of carbohydrates, 15.9 g of protein, and 15.7 g of fat) in order to achieve a near-equal carbohydrate content to BOOST<sup>®</sup>. Patients on pancreatic enzyme replacement therapy were instructed to take their normal morning dose with the meal stimulus ( $n = 5$ ). Blood samples were drawn at -10, 0, 15, 30, 60, 90, and 120 minutes during the MMTT for assessment of glucose and C-peptide. On the day of the MMTT also height and weight (to calculate body mass index [BMI]), glycated hemoglobin (HbA1c), and serum creatinine were assessed. MMTTs were included for this study if the recipient had ingested a total volume of 360 mL of Boost<sup>®</sup> or 270 mL of Nutridrink<sup>®</sup> (Figure S1).

### 2.3 | Laboratory assessment

Glucose was measured by the hexokinase method on the COBAS 8000 (Hoffman-La Roche<sup>®</sup>, Basel, Switzerland). C-peptide was measured by a sandwich immunoassay (Roche<sup>®</sup>) with an IMMULITE 2000 XPi analyzer (Siemens<sup>®</sup>, Munich, Germany) with a lower detection limit of 0.03 nmol/L.

### 2.4 | Statistical analysis

Data are described as mean  $\pm$  SD unless stated differently. For fasting glucose and C-peptide the samples taken at  $t = 0$  minute of the MMTT were used. Peak C-peptide was defined as the highest plasma C-peptide found during the 2-hour MMTT. The area under the curve (AUC) C-peptide was calculated by dividing the AUC of the C-peptide curve (measured by the trapezoid rule) by the total time (120 minutes).

Because most included patients underwent more than one MMTT, mixed models were used with patient identity as random intercept for all analyses to correct for repeated measures within one individual. Linear mixed models were used to investigate whether

stimulated  $\beta$  cell function could be estimated from fasting indices. If C-peptide was undetectable at all time points during the MMTT, the MMTT was excluded from the linear mixed model. MMTTs were also removed from the linear mixed model estimating AUC C-peptide if there was a missing value of C-peptide at one of the time points. When a model was nested, the goodness of fit were compared using the likelihood-ratio test. To investigate how our model performs in comparison with other known indices, we also estimated the peak C-peptide and AUC C-peptide using the Secretary Unit of Islet Transplant Objects (SUITO) index,<sup>10</sup> C-peptide/glucose ratio (CP/G),<sup>11</sup> C-peptide/glucose creatinine ratio (CP/GC)<sup>11</sup> and the beta-2 score.<sup>12</sup> We then compared the result with our model using  $R^2$ , the Akaike information criterion (AIC), and the Bayesian information criterion (BIC). To investigate whether the type of liquid meal, BMI, weight, kidney function, or the use of basal insulin had any influence on the estimation of peak C-peptide or AUC C-peptide, a mixed linear model was used with fasting glucose, fasting C-peptide, and the parameter of interest. Fasting C-peptide, peak C-peptide, and AUC C-peptide were logarithmically transformed in the linear models because of heteroscedasticity (Figure S2). The beta-2 score, SUITO index, CP/G, and CP/GCR were logarithmically transformed to conform normality. All analyses were performed using STATA version 14.1 (StataCorp LP, College Station, TX) and for all analyses a  $P < .05$  was considered statistically significant.

### 3 | RESULTS

#### 3.1 | Islet transplant recipient and mixed meal tolerance test characteristics

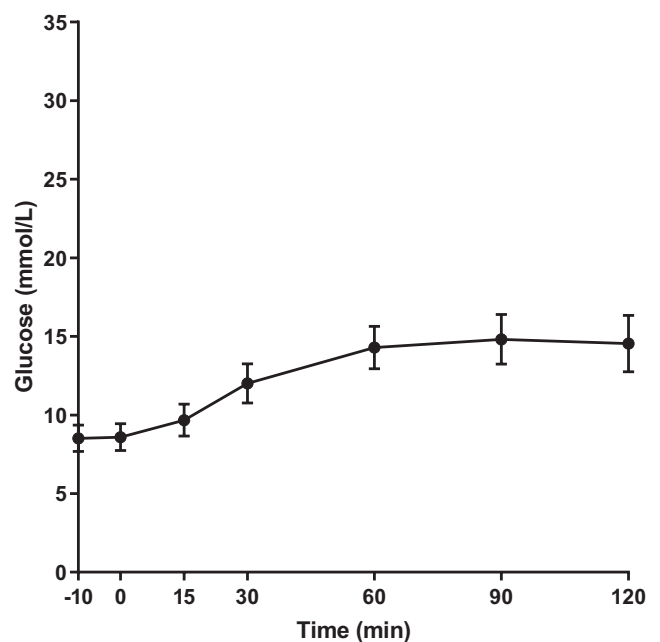
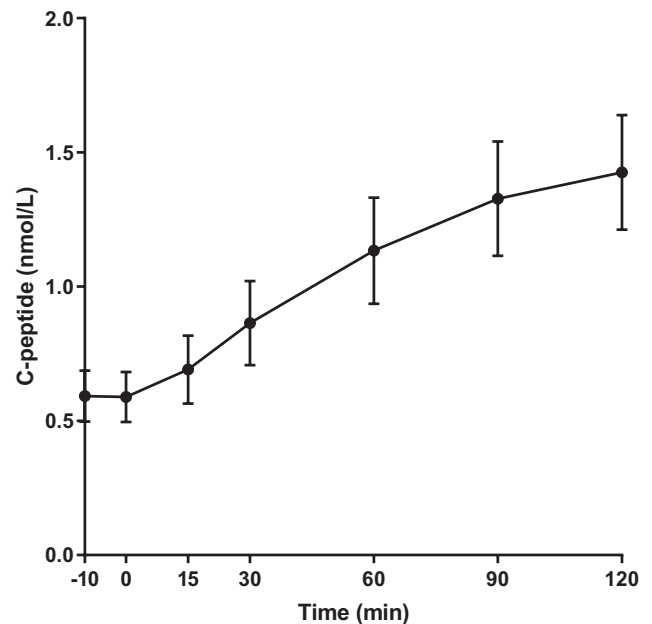
Thirty-six islet transplant recipients (44.4% female, age  $53.5 \pm 10.1$  years, BMI  $22.3 \pm 3.8$  kg/m<sup>2</sup>) were included, of whom 27

**TABLE 1** Baseline characteristics of islet transplant recipients at the first mixed meal tolerance test

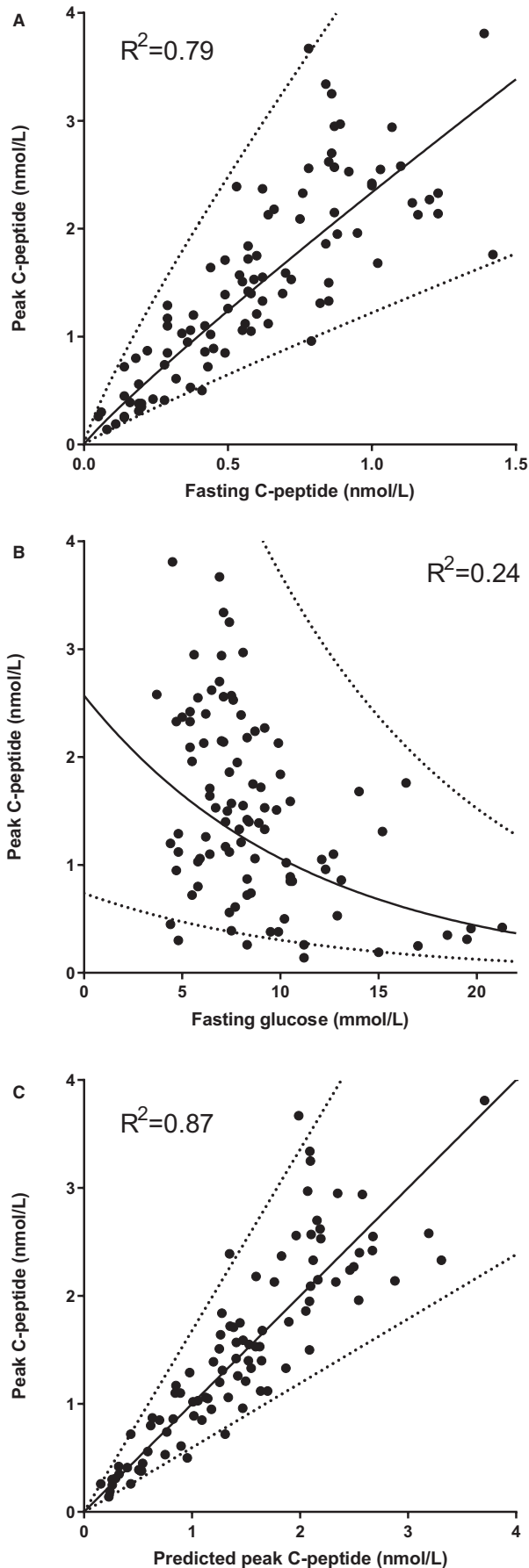
Patients (n)	36
Sex (male/female)	20/16
Age (y)	53.5 (10.1)
Body mass index (kg/m <sup>2</sup> )	22.3 (3.8)
Diabetes duration (y)	33.7 (14.5)
HbA1c (%/mmol/mol Hb)	6.5 (1.2)/47.6 (13.2)
Estimated glomerular filtration rate (mL/min/1.73 m <sup>2</sup> )	57.7 (20.8)
Type of transplantation	
Islet after kidney transplantation	27
Islet transplantation alone	4
Islet after lung transplantation	2
Islet autotransplantation after total pancreatectomy	3

Note: Continuous data is reported as mean (SD) when not stated differently.

subjects had received an islet-after-kidney (IAK) transplantation, 4 subjects an islet transplantation alone (ITA), 2 subjects an islet-after-lung (IAL) transplantation (both patients with cystic fibrosis), and 3 subjects a total pancreatectomy followed by an islet autotransplantation (TP-IAT) (Table 1). At inclusion 21 recipients had received 1 islet graft, 8 recipients had received 2 islet grafts, and 7 recipients had received 3 or more islet grafts with cumulatively  $1\,086\,945 \pm 502\,238$  islet equivalents (IEQ) per recipient. During the study period, some recipients received (an) additional islet graft(s) (Table S1).



**FIGURE 1** Mean C-peptide (upper panel) and mean glucose (lower panel) during the MMTT. At T = 0 min the mixed meal was administered. The error bars represent 95% confidence intervals



**FIGURE 2** Estimation of peak C-peptide by three mixed models: univariate model with fasting C-peptide (A), univariate model with fasting glucose (B), and multivariate model with both fasting C-peptide and fasting glucose (C). All three models had patient identity as random effect. The individual observations are depicted by dots, the fitted model by the continuous line and the 95% estimation interval by the dashed lines. Fasting C-peptide and peak C-peptide were logarithmically transformed before they were put in the model and back-transformed afterwards to present absolute values in the figures

These 36 recipients underwent 120 MMTT of which 100 MMTT were eligible for analysis (Figure S1). On average  $2.8 \pm 1.5$  MMTTs per recipient were included (1 MMTT in 8 recipients, 2 MMTTs in 10 recipients, 3 MMTTs in 8 recipients, 4 MMTTs in 4 recipients, 5 MMTTs in 4 recipients, and 6 MMTTs in 2 recipients). The estimated glomerular filtration rate differed between the transplantation groups (IAK  $54.9 \pm 21.6$  mL/min/1.73 m<sup>2</sup>, ITA  $61.5 \pm 18.1$  mL/min/1.73 m<sup>2</sup>, IAL  $67.8 \pm 25.6$  mL/min/1.73 m<sup>2</sup>, and TP-IAT  $88.9 \pm 6.0$  mL/min/1.73 m<sup>2</sup>).

Sixty-one MMTTs were performed without concurrent use of exogenous insulin, of which 14 MMTTs in insulin-independent recipients and 47 MMTTs in recipients in which basal insulin was temporarily stopped for the test. During 31 MMTTs recipients used CSII at their normal basal rate ( $n = 7$ ) or at 50% of their normal basal rate ( $n = 3$ ), or long-acting insulin at their normal dose ( $n = 15$ ) or a reduced dose (median dose: 65% of normal, range: 58%-93%;  $n = 6$ ). In 8 MMTTs insulin use during the test could not be established from the available data.

### 3.2 | Insulin secretion during the mixed meal tolerance test

In 95 of 100 MMTTs, C-peptide was detectable at all time points. During these 2-hour MMTTs C-peptide increased from 0.59 (95% [confidence interval] CI: 0.50-0.68) to a peak of 1.49 (95% CI: 1.27-1.71) nmol/L with corresponding glucose concentrations of 8.5 (95% CI: 7.7-9.4) and 16.1 (95% CI: 15.5-17.6) mmol/L (Figure 1). No C-peptide was detectable at any time points in the other five MMTTs. There were no MMTTs in which detectable peak C-peptide was observed at later time points after a nondetectable fasting C-peptide. No further MMTTs were performed in patients with nondetectable C-peptide during the MMTT.

### 3.3 | Estimating stimulated $\beta$ cell function with fasting C-peptide and fasting glucose

Fasting C-peptide was strongly and positively correlated with peak C-peptide ( $R^2 = .79$ ,  $P < .0001$ ) and fasting glucose was inversely correlated with peak C-peptide to a much smaller extent ( $R^2 = .24$ ,  $P < .0001$ ) in univariate analysis (Figure 2 and Table 2). The combination of fasting C-peptide and glucose provided the best estimation

of peak C-peptide in the multivariate analysis ( $R^2 = .87, P < .0001$  vs both univariate models). The formula describing this model is:

$$\text{Peak C-peptide (120minutesMMTT)} = e^{1.295 + 0.840 \cdot \ln(\text{fasting C-peptide}) - 0.058 \cdot \text{fasting glucose}}$$

Using AUC C-peptide as outcome parameter, fasting C-peptide was again positively correlated ( $R^2 = .90, P < .0001$ ) and fasting glucose was inversely correlated ( $R^2 = .21, P = .0001$ ) in univariate analysis (Figure 3 and Table 2). Likewise, the combination of fasting glucose and C-peptide provided the best estimation of AUC C-peptide in a multivariate model ( $R^2 = .93, P < .0001$  vs both univariate models). The formula describing this model is:

$$\text{AUC C-peptide (120minutesMMTT)} = e^{0.900 + 0.936 \cdot \ln(\text{fasting C-peptide}) - 0.041 \cdot \text{fasting glucose}}$$

Being an islet allograft or islet autograft recipient did not show interaction with both the association of fasting glucose and fasting C-peptide on peak C-peptide ( $P = .55$  and  $P = .43$ , respectively) and AUC C-peptide ( $P = .91$  and  $P = .73$ , respectively). When removing the 8 MMTTs that were performed in the islet autograft recipients, the models changed only minimally (Table S2).

### 3.4 | Comparison with other known indices

To compare our models with known indices of graft function on accuracy of predicting stimulated  $\beta$  cell function, we estimated peak C-peptide and AUC C-peptide using the SUIITO index, CP/G, CP/GCR, and the beta-2 score. All these indices were also moderately to well associated with peak C-peptide ( $R^2$  ranging from .67 to .85) and AUC C-peptide ( $R^2$  ranging from .71 to .89), whereas our model could estimate peak C-peptide with  $R^2 = .87$  and AUC C-peptide with  $R^2 = .93$  (Table 3).

### 3.5 | Effect of insulin treatment on stimulated $\beta$ cell function

To investigate the effect of basal insulin use on stimulated  $\beta$  cell function, we compared the results of the 92 MMTTs in which insulin use was documented. The MMTTs were subdivided in MMTTs during which no insulin (NI) was used ( $n = 61$ ) and MMTTs in which basal insulin (BI) was continued ( $n = 31$ ). The NI group was further subdivided in MMTT in insulin-independent recipients (NI-II,  $n = 14$ ) and MMTT during which insulin was temporarily stopped (NI-IS,  $n = 47$ ) (Figure 4).

Age, HbA1c, and time since last transplantation were statistically different between the 3 groups with the highest values in the BI group and lowest in the NI-II group (Table 4). In post hoc analysis, only the BI group was statistically significantly different from the other 2 groups (Table S3). BMI and weight also showed the highest values in the BI group and lowest in the NI-II group, but the overall difference did not reach statistical significance ( $P = .06$  and  $P = .08$  respectively). The beta-2 score was clearly associated with the different groups. The NI-II group had the highest and the BI group the lowest beta-2 score ( $P < .001$ ).

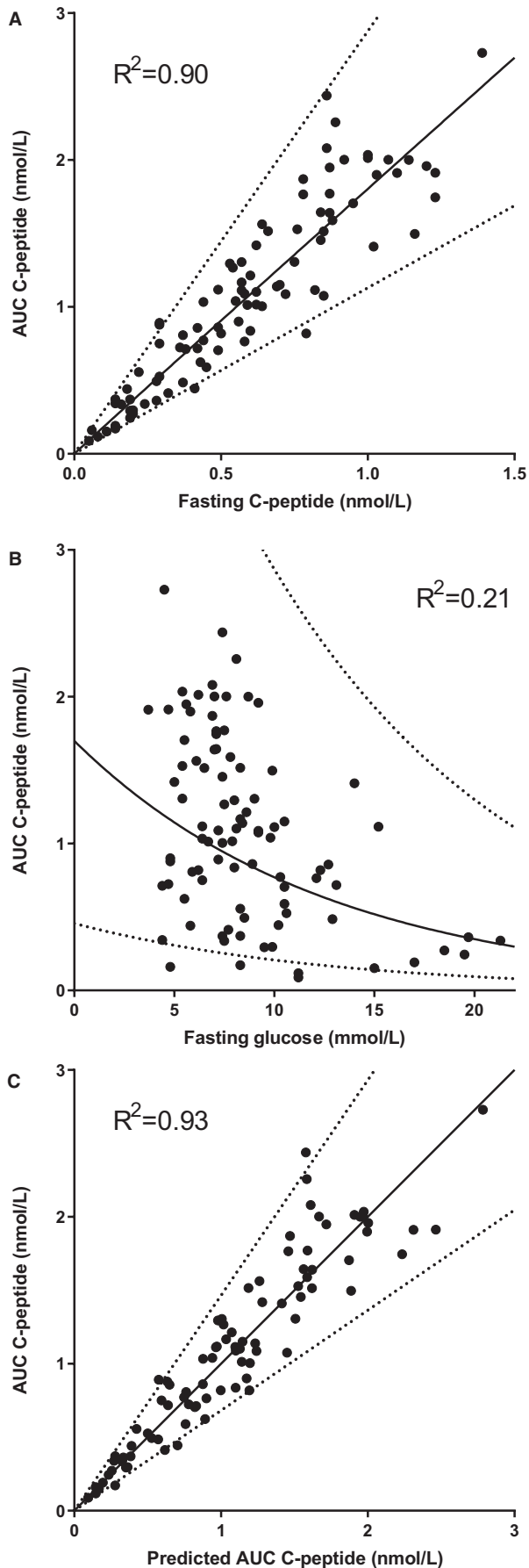
Undetectable C-peptide was only seen in the MMTTs during which insulin was continued ( $n = 4$ ). The other MMTTs in the BI group showed the lowest peak C-peptide (0.92 nmol/L), AUC C-peptide (0.68 nmol/L) and delta AUC C-peptide (0.27 nmol/L) ( $P < .001$  for all comparisons, Figure 4). These lower C-peptide values were observed despite the glucose (8.9 mmol/L) and peak glucose concentration (17.5 mmol/L) being comparable to the NI-IS group ( $P = .63$  and  $P = .34$  respectively) and higher than the NI-II group ( $P = .003$  and  $P = .001$  respectively). The delta glucose in the BI group at the end of the test was even the highest of the three groups (8.7 mmol/L,  $P < .001$  vs NI-IS and  $P = .003$  vs NI-II, Figure 4C).

**TABLE 2** Estimation of both peak C-peptide and AUC C-peptide with fasting glucose and fasting C-peptide

	Peak C-peptide (nmol/L)			AUC C-peptide (nmol/L)		
	Relative change	95% CI	$R^2$	Relative change	95% CI	$R^2$
Univariate analysis						
Fasting C-peptide (per 100% increase)	+91%	+82%, +101%	.79	+99%	+92%, +106%	.90
Fasting glucose (per 1 mmol/L increase)	-8%	-12%, -5%	.24	-8%	-11%, -4%	.21
Multivariate analysis						
Fasting C-peptide (per 100% increase)	+84%	+76%, +92%	.87	+94%	+88%, +100%	.93
Fasting glucose (per 1 mmol/L increase)	-6%	-7%, -4%		-4%	-5%, -3%	

Note: Note. Peak C-peptide, AUC C-peptide, and fasting C-peptide were logarithmically transformed because of heteroscedasticity. For fasting glucose, the effect sizes were transformed back, resulting in a relative change of the outcome variable per unit of glucose. For fasting C-peptide, the relative change of the outcome variable is reported per 100% increase (or doubling) of the fasting C-peptide.

Abbreviations: AUC, area under the curve; CI, confidence interval; Ln, natural logarithm.



**FIGURE 3** Estimation of area under the curve (AUC) C-peptide by three mixed models: univariate model with fasting C-peptide (A), univariate model with fasting glucose (B), and multivariate model with both fasting C-peptide and fasting glucose (C). All three models had patient identity as random effect. The individual observations are depicted by dots, the fitted model by the continuous line and the 95% prediction interval by the dashed lines. Fasting C-peptide and AUC C-peptide were logarithmically transformed before they were put in the model and back-transformed afterwards to present absolute values in the figures

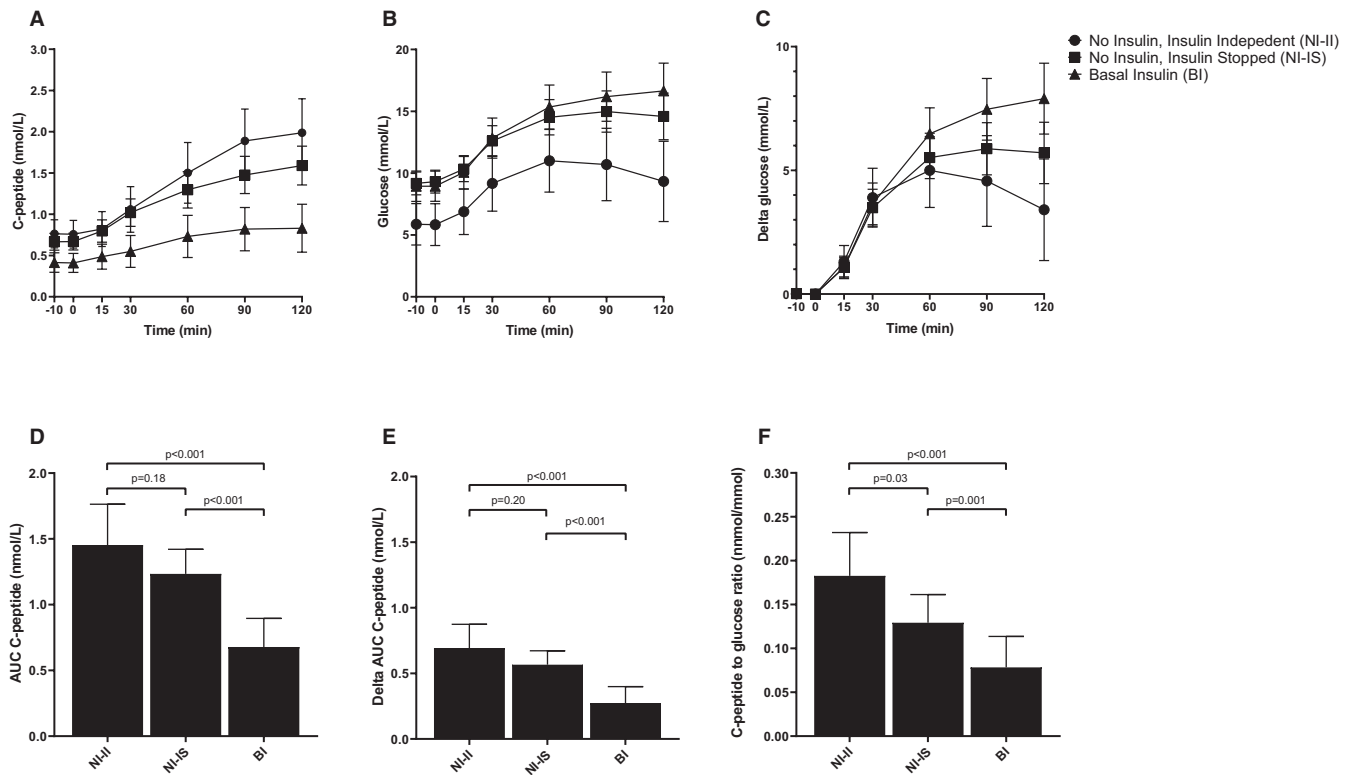
The highest peak C-peptide (2.06 nmol/L) was present in the NI-II group (Figure 4A). Although the peak C-peptide was numerically lower in the NI-IS group (1.67 nmol/L), the difference with the NI-II group did not reach statistical significance ( $P = .11$ ). There was also no statistically different AUC C-peptide (1.45 vs 1.23 nmol/L,  $P = .18$ ) and delta AUC C-peptide (0.69 vs 0.57 nmol/L,  $P = .20$ ) between the NI-II and NI-IS group (Figure 4D,E). The C-peptide concentrations in the NI-IS group were attained in the presence of higher fasting glucose and maximal glucose concentrations compared to the NI-II group (fasting: 9.3 vs 5.8 mmol/L,  $P < .001$ , maximal: 16.4 vs 11.7 mmol/L,  $P = .005$ ) (Figure 4B). As a consequence, C-peptide/glucose ratio at  $t = 90$  minutes, as another measure of  $\beta$  cell function, was decreased in the NI-IS group compared to the NI-II group (0.13 vs 0.18 nmol/mmol,  $P = .03$ ) (Figure 4F).

To determine the effect of exogenous insulin use on stimulated  $\beta$  cell function under the same basal conditions, exogenous insulin use was added to the mixed model together with fasting glucose and fasting C-peptide (Table 5). With the same fasting C-peptide and fasting glucose, basal insulin use was still associated with a significant lower peak C-peptide (relative change:  $-15\%$ , 95% CI:  $-26\%$ ,  $-3\%$ ), when compared to the MMTTs during which no insulin was used (NI-II and NI-IS groups combined). There was no such difference in estimated peak C-peptide under the same fasting glucose and fasting C-peptide, when comparing the NI-II and NI-IS groups ( $P = .92$ ).

**TABLE 3** Estimation of both peak C-peptide and area under the curve (AUC) C-peptide using different indices

	Predicting peak C-peptide			Predicting AUC C-peptide		
	$R^2$	AIC	BIC	$R^2$	AIC	BIC
Beta-2 score	.69	84.7	94.4	.71	78.1	87.6
SUITO	.70	103.9	114.1	.71	99.8	109.8
CP/G	.85	38.7	48.9	.89	15.3	25.3
CP/GCR	.67	93.0	103.2	.73	71.4	81.4
Our model	.87	26.9	39.7	.93	-25.3	-16.7

Abbreviations: AIC, Akaike information criterion; BIC, Bayesian information criterion; CP/GCR, C-peptide/glucose creatinine ratio; CP/G, C-peptide/glucose ratio; SUITO, Secretory Units of Islet Transplant Objects.



**FIGURE 4** The upper part of the figure shows the C-peptide concentration (A), glucose concentration (B), and delta glucose concentration (C) during the mixed meal tolerance test (MMTT). The P values are for differences in the whole curve between the groups (time series analysis with a mixed model). The lower part of the figure shows the area under the curve (AUC) C-peptide (D), delta AUC C-peptide (E), and the C-peptide to glucose ratio (F) during the MMTT. T = 0 is defined as the moment the meal test was given. The error bars represent 95% confidence intervals. BI, basal insulin (n = 31); NI-II, no insulin, insulin independent (n = 14); NI-IS, no insulin, insulin stopped (n = 47)

**TABLE 4** Clinical characteristics of the islet transplant recipients grouped by insulin use

	No insulin–insulin independent (NI-II)	No insulin–insulin stopped (NI-IS)	Basal insulin (BI)	P value*
Mixed meal tolerance tests (N)	14	47	31	
Age (y)	53.4 (49.8, 57.0)	54.1 (50.6, 57.5)	55.3 (51.8, 58.7)	.004
Weight (kg)	65.6 (60.4, 70.8)	67.0 (62.2, 71.8)	68.3 (63.4, 73.2)	.08
Body mass index (kg/m <sup>2</sup> )	22.0 (20.5, 23.4)	22.4 (21.2, 23.6)	22.9 (21.7, 24.1)	.06
Insulin dose (IU/kg)	–	0.35 (0.28, 0.41)	0.42 (0.35, 0.49)	.055
HbA1c (%) (mmol/mol Hb)	5.8 (5.0, 6.6) 40.0 (31.4, 48.6)	6.4 (5.9, 6.8) 45.9 (41.0, 50.8)	7.8 (7.3, 8.3) 61.5 (55.8, 67.2)	<.001
Time since last transplantation (y)	1.1 (0.01, 2.2)	1.3 (0.6, 2.0)	2.6 (1.9, 3.4)	<.001
Beta-2 score	23.2 (18.0, 28.4)	14.9 (11.6, 18.3)	9.6 (5.9, 13.3)	<.001

Note: The data are presented as mean (95% CI).

\*P value for a difference between the 3 groups (analysis of variance-style test in the mixed model). The intergroup differences are shown in Table S2.



**TABLE 5** The influence of clinical parameters on peak and area under the curve (AUC) C-peptide

	Peak C-peptide			AUC C-peptide		
	Relative change	95% CI	P value	Relative change	95% CI	P value
BI vs NI-IS + NI-II	-15%	-26%, -3%	.02	-10%	-19%, 0%	.050
NI-IS vs NI-II	+1%	-14%, +18%	.92	0%	-12%, +14%	.98
Nutridrink® (vs Boost®)	-3%	-14%, +9%	.62	+1%	-8%, +10%	.87
Length (per cm)	-0.2%	-0.9%, +0.4%	.61	-0.3%	-0.9%, +0.2%	.19
Weight (per kg)	0%	-0.4%, +0.5%	.86	0%	-0.3%, +0.3%	.96
Body mass index (per kg/m <sup>2</sup> )	+0.4%	-1.2%, +2.0%	.63	+0.4%	-0.9%, +1.6%	.56
HbA1c (per %)	-5.1%	-8.9%, -1.2%	.01	-5.4%	-8.3%, -2.4%	<.001
(per mmol/mol Hb)	-0.5%	-0.9%, -0.1%		-0.5%	-0.8%, -0.2%	
eGFR (per mL/min/1.73 m <sup>2</sup> )	+0.1%	-0.2%, +0.3%	.50	+0.1%	-0.1%, +0.3%	.23
Time since last transplantation (per y)	-1.5%	-4.2%, +1.3%	.28	-1.1%	-3.2%, +1.0%	.29

Note: The influence of clinical parameters on the peak C-peptide and AUC C-peptide was investigated by adding the variable of interest to the mixed model together with fasting C-peptide and fasting glucose as independent variables. Peak C-peptide, AUC C-peptide and fasting C-peptide were logarithmically transformed, because of heteroscedasticity. The effect sizes were transformed back, which means reporting in relative change of the estimated variable per unit of the input variable.

Abbreviations: BI, basal insulin; eGFR, estimated glomerular filtration rate; NI-II, no insulin, insulin independent; NI-IS, no insulin, insulin stopped.

### 3.6 | Effect of other factors on stimulated $\beta$ cell function

HbA1c had a statistically significant effect on peak C-peptide (relative change per % HbA1c: -5.1%, 95% CI: -8.9%, -1.2%) and AUC C-peptide (relative change per % HbA1c: -5.4%, 95% CI: -8.3%, -2.4%) in a multivariate model with fasting glucose and fasting C-peptide (Table 5). Length, weight, BMI, and estimated glomerular filtration rate were not associated with peak C-peptide and AUC C-peptide independently from fasting C-peptide and fasting glucose. In the recipients who had received Nutridrink®, weight was also not associated with peak C-peptide and AUC C-peptide when MMTTs in recipients <60 kg only were analyzed (N = 26, P = .50 and P = .56, respectively).

When adjusting for the fasting glucose and fasting C-peptide using the linear mixed model, there was no effect of Nutridrink® when compared to Boost® on the peak C-peptide (relative change: -3%, 95% CI: -14%, +9%) and the AUC C-peptide (relative change: +1%, 95% CI: -8%, +10%) (Table 5).

### 3.7 | Use of MMTT and IglS criteria for graft outcome

The IglS criteria require a C-peptide >0.17 nmol/L, fasting or stimulated, for functional beta-cell graft status. To assess the clinical usefulness of the final model, we investigated how well our model predicts peak C-peptide >0.17 nmol/L when fasting C-peptide is  $\leq$ 0.17 nmol/L, thereby avoiding the need of a stimulation test. In 86 of the 100 MMTTs, the fasting C-peptide was already >0.17 nmol/L. In 5 of the remaining tests, fasting C-peptide was undetectable (detection limit 0.03 nmol/L) and in all of these MMTTs C-peptide

remained undetectable during the MMTT. In the final 9 MMTTs the fasting C-peptide was between 0.05 and 0.16 nmol/L (mean:  $0.11 \pm 0.04$  nmol/L) and the peak C-peptide between 0.14 and 0.72 (mean:  $0.33 \pm 0.17$ ). In 7 out of these 9 tests the model classified peak C-peptide >0.17 nmol/L correctly.

## 4 | DISCUSSION

Standardized  $\beta$  cell stimulation tests are performed when assessing and monitoring graft function after islet transplantation. However, these tests are time consuming and cumbersome, leading to infrequent testing. Our main results show that by using fasting C-peptide and glucose concentrations, we were able to reliably estimate peak C-peptide and AUC C-peptide in islet transplant recipients with and without concurrent insulin treatment. Using only fasting indices to estimate stimulated C-peptide is a viable option for more frequent monitoring of graft function. In this way, graft deterioration and thereby acute rejection may be picked up earlier with a larger window of opportunity to intervene.

We focused specifically on stimulated  $\beta$  cell function because it is least influenced by recipient-related factors. Other indices such as the  $\beta$ -score, the beta-2 score, the SUIITO index and the transplant estimated function (TEF) are more focused on clinical outcomes such as insulin independence and glucose values after a stimulation test.<sup>10-12,14,15</sup> Our model was specifically developed for estimating stimulated  $\beta$  cell function using fasting glucose and fasting C-peptide and therefore performed very well in estimating  $\beta$  cell function. Because the fasting C-peptide/glucose ratio uses the same parameters as our model, it is not surprising that this parameter also performed well. Naturally, the aforementioned other indices and indicators of glycemic control are very useful when assessing (clinical) transplantation outcome, and we

therefore regard our model as an addition and not a replacement of existing indices and relevant indicators of glycemic control.

Estimating stimulated  $\beta$  cell function with fasting parameters has been investigated in patients with recent-onset type 1 diabetes. In an analysis of multiple TrialNet studies, the average C-peptide concentration during a 120-minute MMTT was estimated with a similar model using fasting C-peptide, fasting glucose, BMI, disease duration, insulin dose, and HbA<sub>1c</sub> with a  $R^2$  of .816.<sup>16</sup> Another study in patients with recent onset type 1 diabetes also found a correlation between fasting C-peptide and AUC C-peptide ( $R^2 = .71$ ).<sup>17</sup> We found stronger correlations between fasting indices and stimulated  $\beta$  cell function, in spite of the presence of several additional modulating factors in our patient population (such as the use of different immunosuppressive drugs that can affect  $\beta$  cell function). This could be due to a larger range of C-peptide concentrations in our cohort (leading to better estimation) and using logarithmic transformation.

It should be noted that our MMTT was limited to 120 minutes. In 59 out of 100 the peak C-peptide was at 120 minutes. So it cannot be excluded that even a higher peak could be beyond the 120-minute time point. However, the relative rise in C-peptide in those 59 MMTTs was very small between 90 and 120 minutes (+14%); thus, it is most likely that the peak was present at 120 minutes or that the further increase would be even smaller and would not have a major impact on our results. For feasibility purposes, most centers perform 90- or 120-minute MMTTs enabling the use of and comparisons with our model.<sup>11,15,18,19</sup>

Adding fasting glucose to fasting C-peptide greatly improved the accuracy of the estimation of stimulated C-peptide in our study; a higher fasting glucose was consistently associated with lower peak C-peptide and AUC C-peptide. In contrast to our findings, a study found a higher incremental AUC C-peptide after glucose was acutely raised by glucose infusion just before the MMTT.<sup>20</sup> This could be explained by our patients having a much longer duration of elevated baseline glucose when they arrived for the MMTT and chronic hyperglycemia is known to have detrimental effect on  $\beta$  cell function (glucose toxicity).<sup>21,22</sup> In our study, the causality is probably also partly reversed, indicating that patients with worse (stimulated)  $\beta$  cell function are more likely to arrive with higher fasting glucose.

In the group that used basal insulin during the MMTT, there was a higher maximum glucose concentration and a larger increase of glucose during the MMTT. However, the use of basal insulin during the MMTT was associated with lower stimulated C-peptide, even when corrected for fasting glucose and fasting C-peptide. The most likely explanation is that the use of basal insulin during the test is an indicator of a graft with poorer function, which notion is further strengthened by the lower beta-2 score in this group.

There was no statistically significant association between weight or BMI and stimulated C-peptide parameters. Because ingestion of less than 360 mL of Boost<sup>®</sup> was an exclusion criterium in the study, no MMTTs from recipients <60 kg with Boost<sup>®</sup> as the stimulus were included. Because there was no weight adjustment for the amount of Nutridrink<sup>®</sup> stimulus, in this subgroup MMTTs from recipients

<60 kg were also included and no association between weight and stimulated C-peptide was observed. Therefore, our data do not support weight adjustment for the amount of Nutridrink<sup>®</sup> in an adult population, as is usual with BOOST<sup>®</sup>.

A potential limitation with regard to application of our model in the transplantation clinic is the necessity that patients must arrive in a fasting state. Patients in whom insulin cannot be stopped must be carefully evaluated before the mixed meal test to decide whether an insulin dose adjustment is necessary to prevent hypoglycemia. Hypoglycemia followed by ingestion of carbohydrates in the hours before the MMTT requires MMTT rescheduling, which is a burden to the patient and health care providers. Nonfasting C-peptide may be a reliable proxy for a standardized stimulation test. A study using nonfasting C-peptide (within 5 hours of a meal) to estimate 90-minute MMTT C-peptide in patients with type 2 diabetes found a correlation of  $R^2 = .83$  and noted that the reliability would go up to  $R^2 = .92$  when a nonfasting C-peptide with a concurrent glucose concentration of  $\geq 8$  mmol/L were used.<sup>23</sup> A model using nonfasting C-peptide in islet cell recipients may therefore be possible, if corrected for the concurrent glucose.

In conclusion, we found that peak C-peptide and AUC C-peptide could be accurately estimated by fasting C-peptide and fasting glucose in islet transplantation recipients both with and without basal insulin use. This knowledge can be used to save costs and to assess  $\beta$  cell function more frequently. This could potentially lead to earlier detection of a decline in  $\beta$  cell function and a larger window of opportunity for intervention.

## DISCLOSURE

The authors of this manuscript have no conflicts to disclose as described by the *American Journal of Transplantation*.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

## ORCID

Bas S. Uitbeijerse  <https://orcid.org/0000-0003-4574-6722>

Michiel F. Nijhoff  <https://orcid.org/0000-0002-5131-2237>

Jacob K. Sont  <https://orcid.org/0000-0002-5840-0651>

Elco J. P. de Koning  <https://orcid.org/0000-0002-1232-7022>

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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