A Prospective Study of Renal Transplant Recipients: A Fall in Insulin Secretion Underpins Dysglycemia After Renal Transplantation

David Langsford, MBBS, FRACP,[1,2,3] Varuni Obeyesekere, BSc,[4] Sara Vogrin, MBBS,[3]
Jessie Teng, MBBS, FRACP,[4] Richard J. MacIsaac, MBBS, PhD, FRACP,[3,4] Glenn Ward, MBBS, DPhil,[3,4]
Frank Alford, MBBS, MD, FRACP,[3,4] and Karen M. Dwyer, MBBS, PhD, FRACP[1,3,5,6]

Background. Dysglycemia (encompassing impaired glucose tolerance and diabetes mellitus) arising after renal transplantation is common and confers a significant cardiovascular mortality risk. Nonetheless, the pathophysiology of posttransplant dysglycemia is not well described. The aim of this study was to prospectively and comprehensively assess glucose handling in renal transplant recipients from before to 12 months after transplantation to determine the underpinning pathophysiology. **Materials and Methods.** Intravenous and oral glucose tolerance testing was conducted before and at 3 and 12 months posttransplantation. An intravenous glucose tolerance test was also performed on day 7 posttransplantation. We followed up 16 transplant recipients for 3 months and 14 recipients for 12 months. Insulin secretion, resistance and a disposition index (DI (IV)), a measure of β cell responsiveness in the context of prevailing insulin resistance, were also determined. **Results.** At 12 months, 50% of renal transplant recipients had dysglycemia. Dysglycemia was associated with a dramatic fall in DI (IV) and this loss in β cell function was evident as early as 3 months posttransplantation (23.5 pretransplant; 6.4 at 3 months and 12.2 at 12 months posttransplant). Differences in the β cell response to oral glucose challenge were evident pretransplant in those destined to develop dysglycemia posttransplant (2-hour blood glucose level 5.6 mmol/L versus 6.8 mmol/L; $P < 0.01$). **Conclusions.** Dysglycemia after renal transplantation is common, and the loss of insulin secretion is a major contributor. Subclinical differences in glucose handling are evident pretransplant in those destined to develop dysglycemia potentially heralding a susceptible β cell which under the stressors associated with transplantation fails.

(*Transplantation Direct* 2016;2: e107; doi: 10.1097/TXD.0000000000000618. Published online 7 October, 2016.)

Diabetes mellitus and impaired glucose tolerance (IGT) after renal transplantation are associated with an increased risk of mortality and major cardiovascular events.[1-5] These dysglycemic states may be preexisting or arise de novo after transplantation. Diabetes first diagnosed posttransplantation has previously been called new-onset diabetes after transplantation; however, an international consensus recently recommended the use of the term posttransplant diabetes mellitus (PTDM)[6] to describe diabetes after transplantation. This is due to the difficulty in identifying those recipients with unrecognized diabetes pretransplantation.[7-10] Importantly, the consensus group also acknowledged that further research into the pathophysiology of PTDM was essential and that it should be considered a distinct pathological and clinical entity from type 1 or type 2 diabetes.

Dysglycemia after transplantation (encompassing PTDM and IGT) often develops within the first year posttransplantation; however, the true prevalence is difficult to ascertain because few studies use established diagnostic criteria for diabetes and fewer still the criterion standard oral glucose tolerance test (oGTT).[11] A large proportion of dysglycemia is detectable within the first 3 to 6 months posttransplantation[10,12,13] coinciding with the time of greatest immunological risk and

immunosuppressive burden. Calcineurin inhibitors (CNI) and glucocorticoids form the backbone of most immunosuppressive regimens and are well described risk factors for posttransplant dysglycemia.[14,15] The diabetogenic potential of glucocorticoids is well established and encompasses both increased insulin resistance and reduced insulin secretion. In addition, CNI reduce insulin secretion through a direct toxic effect on the β cell.[16] Balancing the immunological with the metabolic risks by modifying CNI levels and/or ceasing glucocorticoids within the first year posttransplantation remains a challenge. The early identification of those recipients at higher risk of developing a posttransplant dysglycemic state may assist with pretransplantation planning for the prevention of dysglycemia in a manner that would not compromise the graft immunologically. A proof of concept of such prevention of dysglycemia has been described by Hecking et al.[17]

The objectives of this study were two-fold: First, to examine the changes in metabolic indices that occur after transplantation to define the underpinning pathophysiology and second, identify factors evident pretransplant that may be predictive of posttransplant dysglycemia. To this end, we undertook a comprehensive assessment of changes in β-cell function and insulin resistance in renal transplant recipients. We used a rigorous testing protocol that included both intravenous GTT (IV GTT) and oGTT to quantitate β-cell function, before transplantation and at 3 and 12 months posttransplantation. IV GTT was also performed day 7 posttransplantation. The data available from these tests allowed us to evaluate insulin secretion and resistance and calculate the disposition index (DI (IV)), a measure of β cell responsiveness in the context of prevailing insulin resistance.[18-20] Finally, by stratifying recipients according to their posttransplant glycemic state we sought to identify deficits in glucose or insulin indices present at study entry that may serve as early markers of those at higher risk of posttransplant dysglycemia.

MATERIALS AND METHODS

Twenty-five patients with end-stage kidney disease (ESKD) from a single hospital network planned for a living (un)related donor transplant were recruited (Figure 1). None of the patients were known to be diabetic at the time of recruitment into the study. The exclusion criteria were inability to participate in the study over 12 months, known diabetes
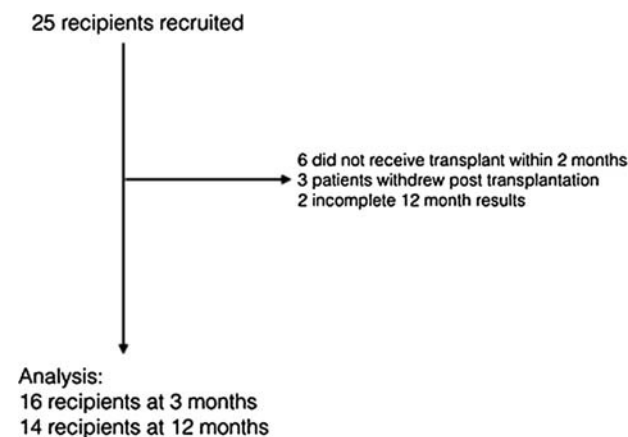

FIGURE 1. Recipient recruitment.

pretransplant or previous transplantation. Six patients did not go onto to receive a transplant within 2 months of testing; 3 withdrew from the study after transplantation. Of the 16 remaining patients, who are referred to as recipients, and 2 recipients had not reached 12 months by the conclusion of the study, leaving 14 recipients with a complete data set. We also recruited 9 potential living kidney donors to undergo single IV GTT and oGTT tests before donation to act as a comparator group. The study was approved by the human ethics committees of the relevant hospitals within the network and signed informed consent was obtained from each participant before commencing the study.

All participants had demographic, BMI, waist-hip ratio, blood pressure and glucometabolic (cholesterol, triglycerides, BSL, HbA1c, oGTT and IV GTT) data collected at baseline. In addition, the renal transplant recipients had renal specific and transplant specific data collected pretransplant and at 3 and 12 months posttransplantation. As outlined in Figure 2, all patients had baseline IV GTT and oGTT tests. Results for glucose and insulin from multiple time points enabled the calculation of glucose metabolism, insulin resistance, insulin secretion and disposition index. Each test was conducted on a different day and after an overnight fast. The renal transplant recipients had these tests conducted within 2 months of renal transplantation. This group also had an IV GTT and oGTT conducted at 3 and 12 months posttransplantation. IV GTT was also performed on day 7 posttransplantation. Any recipient who had been commenced on a hypoglycemic agent had that agent withheld for 72 hours before each test. The oGTT test was conducted by administering 75 g of oral glucose and drawing a venous sample from an intravenous cannula placed in the cubital fossa of the nonarteriovenous fistula arm at 0, 30, 60 and 120 minutes. The IV GTT test was conducted by administering 0.3 mg/kg intravenous glucose to a maximum dose of 25 g, diluted in normal saline to a maximum volume of 100 mL. Venous samples were drawn from an intravenous cannula placed in the cubital fossa of the non-arteriovenous fistula arm at −10, −1, +2, +4, +6, +8, +10, +12, +14, +19, +25, +40 minutes. It has previously been established that this dose of intravenous glucose elicits the maximal pancreatic response over this period[21] and similar protocols have been successfully used.[22]

Glucose metabolism was assessed by 2 different approaches. Firstly, a comprehensive descriptive analysis was aided by the area under the curve (AUC) of oGTT of each recipient at pretransplant, 3 and 12 months posttransplant. Secondly, an improved understanding of the pathophysiology was gained by assessing the first phase insulin release (FPIR), insulin resistance and β cell responsiveness. FPIR is an index of the β cell response to intravenous glucose (insulinogenic index) and was calculated using the IV GTT by FPIR = (AUC insulin t0-10 minutes)/(Glucose t0minutes − (Av(t-10 minutes and t-1 minutes)) as previously described.[23] Insulin resistance was determined using paired insulin and glucose results obtained from IV GTT, which were then applied to the homeostatic model assessment 2 (HOMA2) calculator, available from https://www.dtu.ox.ac.uk/homacalculator/download.php. This has previously been validated in ESKD patients.[24] Insulin sensitivity is the inverse of insulin resistance. The DI (IV) was calculated as the FPIR/insulin resistance. The DI (IV) is a measure of β cell responsiveness and has been shown in the general population to be
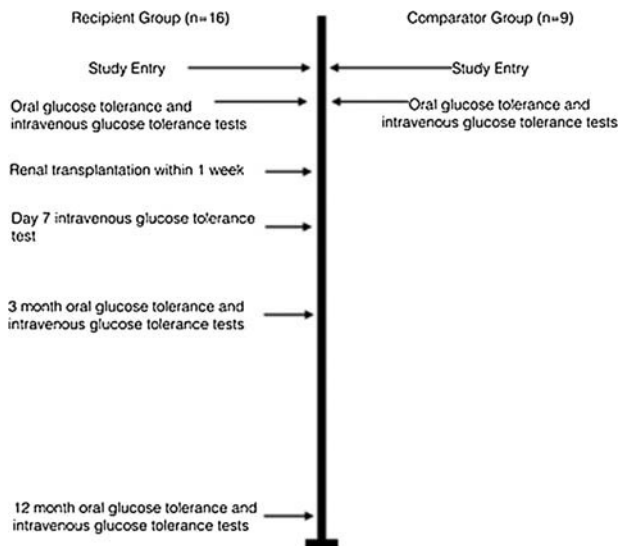
FIGURE 2. Study protocol.

predictive of the development of type 2 diabetes mellitus in those at clinically increased risk.[25] Disposition index relates insulin sensitivity and insulin resistance in a hyperbolic relationship such that an increase an insulin resistance is associated with a compensatory increase in insulin secretion to maintain normoglycemia.[18]

At the time of transplantation, recipients received 20 mg basiliximab on days 0 and 4, 500 mg intravenous methylprednisolone on day 0 followed by 30 mg daily of oral prednisolone which was weaned after 3 weeks by 2.5 mg every 2 weeks to a target dose of 5 mg daily. All recipients received 1 g twice a day of mycophenolate mofetil and a CNI, however, the choice of agent was at the discretion of the treating transplant physician. For those who received cyclosporin, the starting dose was 4 mg/kg twice a day with a C2 target of 1200 for the first month, 800 to 1000 at 3 months and 400 to 600 at 12 months. For those who received tacrolimus, the starting dose was 0.075 mg/kg twice a day with a trough tacrolimus target of 8 to 10 for the first month and 5 to 8 at 3 months. All recipients received protocol cytomegalovirus and *Pneumocystis jiroveci* pneumonia prophylaxis. Renal transplant biopsies were conducted as protocol at 3 months or as indicated by clinical criteria. Acute cellular rejection was treated with oral prednisolone 300, 200, and 100 mg over 3 consecutive days. To comprehensively assess immunosuppression burden weekly therapeutic drug levels for CNI agents were collected and the cumulative exposure determined by calculating the AUC.

Dysglycemia, encompassing IGT and diabetes, was defined using oGTT as the diagnostic test and the World Health Organization diagnostic criteria of 2 hour oGTT result of ≥7.8 mmol/L (140 mg/dl) for impaired glucose tolerance and ≥11.1 mmol/L (200 mg/dl) for diabetes applied.

Statistical Analysis

Data is represented as median and interquartile ranges for continuous variables and as frequencies and percentages for categorical variables. Continuous variables between groups were compared using Kruskal-Wallis and Wilcoxon rank sum test. Categorical variables were compared using Fisher exact test. Significance level of 0.05 was not corrected for multiple comparisons due to explorative nature of the study. All analyses were performed using Stata 13.

RESULTS

Data from 9 healthy donors (forming the comparator group) and sixteen recipients were analyzed. The demographic and routine clinical parameters at entry into the study are outlined in Table 1. All participants were Caucasian. Recipients were well matched to the comparator group with respect to age, sex, waist to hip ratio, body mass index and fasting lipid profile, although systolic blood pressure was significantly lower in the comparator group. The cause of renal failure in the recipients was primarily attributable to glomerulonephritis and 75% of the recipients had already commenced dialysis. All but 1 recipient was cytomegalovirus positive and there were no participants with hepatitis C infection.

Using standard screening tests for dysglycemia of fasting blood glucose level (F BGL) or HbA1c we did not observe any differences between the recipient and comparator groups (Table 2A). However, oGTT testing revealed unrecognized dysglycemia in 3 recipients (Table 2B). All 3 patients were included in the subsequent analysis. In addition, none of the glucometabolic tests as measured by FPIR (insulin secretion), HOMA2IR (insulin resistance), and DI (IV) (β cell responsiveness) aided in discriminating between normoglycemic versus dysglycemic recipients pretransplant (Table 2B), or all recipients versus comparator group (Table 2A).

At 12 months posttransplant 14 recipients had data available for analysis. Seven (50%) recipients had evidence of dysglycemia (Table 3) which was evident only on 2 hour BGL testing. Fasting BGL and HbA1c testing performed poorly as a screening tool in identifying these recipients (all dysglycemic patients had a F BGL < 7 mmol/L and

TABLE 1.

Demographic, renal specific and glucometabolic data at entry into study

Variable at entry into study	Comparator group (n = 9)	All recipients (n = 16)	P
Age	48 (44,54)	42.5 (34, 52.5)	0.223
Sex M/F	6/3	10/6	1.000
ESKD etiology PCKD		3	N/A
Glomerulonephritis		8	
Other		5	
Waist-hip ratio	0.89 (0.83,0.97)	0.90 (0.87, 1.06)	0.439
Body mass index, kg/m²	28.0 (22.1,28.4)	26.8 (24.2, 30.1)	0.624
Blood pressure—systolic	115 (110, 120)	130 (120, 150)	0.019
Blood pressure—diastolic	80 (75, 85)	80 (70, 91.5)	0.605
Total cholesterol, mmol/L	5.5 (5.0, 5.8)	5.0 (4.1, 5.5)	0.105
Total cholesterol, mg/dl	212 (193, 224)	193 (158, 212)	
Triglycerides, mmol/L	1.3 (1.0, 1.4)	1.6 (1.2, 2.2)	0.057
Triglycerides, mg/dl	115 (89, 124)	142 (106, 195)	
Prior hemodialysis		5	N/A
Prior peritoneal dialysis		7	
Preemptive transplantation		4	
Hepatitis C positive		0	
CMV positive		15	N/A

All values expressed as median (25%,75% percentile) unless specified.
Dysglycemia is defined by 2 hour oGTT result of ≥7.8 mmol/L (140 mg/dl).
CMV, cytomegalovirus.

TABLE 2A.

Glucometabolic data at entry in study of comparator versus recipient groups

Variable at entry into study	Comparator (n = 9)	Recipients (n = 16)	P
F BGL, mmol/L	4.9 (4.6, 5.3)	4.5 (4.3, 4.9)	0.174
F BGL, mg/dl	88 (83, 95)	81 (77, 88)	
2-h oGTT, mmol/L	5.4 (4.8, 6.4)	6.3 (5.5, 6.9)#	0.311
2-h oGTT, mg/dL	97 (86, 115)	113 (99, 124)	
oGTT AUC	798 (785, 911)	846 (788, 934)^	0.713
HbA1c%	5.2 (5.0, 5.4)	5.1 (4.8, 5.6)#	0.568
HbA1c, mmol/mol	33.3 (31.1,35.5)	32.2 (29, 37.7)	
HOMA2IR	1.2 (1.2, 1.6)	1.1 (0.8, 1.4)#	0.698
FPIR	29.4 (22.8, 33.3)	38.7 (18.8, 36.7)#	0.531
DI	24.5 (22.7, 40.0)	25.5 (18.8, 28.7)^	0.493

All values expressed as median (25%,75% percentile) unless specified.
Dysglycemia is defined by 2 hour oGTT result of ≥7.8 mmol/L (140 mg/dl).
DI (IV), intravenous disposition index and is a measure of β cell responsiveness.
#1missing value.
^3 missing values.

HbA1c < 6.3%), although the HbA1c was significantly higher in the dysglycemic compared to normoglycemic groups. We observed dysglycemia in recipients on both cyclosporine and tacrolimus. A prior episode of rejection requiring pulse steroids occurred in only one recipient. The most striking feature on detailed analysis was the drop in DI (IV) in the dysglycemic recipients (*P* = 0.007). A modest increase in weight was observed in the 2 groups: normoglycemia median 71 kg (64.5 kg, 81.4 kg 25%-75% quartile) at study entry and 77.7 kg (65.2, 83.5 kg, 25-75% quartile) at 12 months and dysglycemia median 80 kg (75.5 kg, 84.5 kg 25%-75% quartile) at study entry and 83 kg at 12 months (78 kg, 94.1 kg 25% - 75% quartile). Despite these differences in weight the 12 month HOMA2IR measured insulin resistance in both groups was comparable with each other the comparator group. Thus, the reduction in DI (IV) is most consistent with a fall in insulin secretion and β cell function.

TABLE 2B.

Glucometabolic data at entry into study of recipients

Variable at entry into study	Normoglycemic recipients (n = 13)	Dysglycemic recipients (n = 3)	P
Age	46 (34,54)	35 (30, 50)	0.381
Waist-hip	0.9 (0.88, 1.05)	0.86 (0.73, 1.1)	0.545
Body mass index, kg/m²	26.3 (25.3, 30.1)	27.4 (21.8, 30.1)	0.947
F BGL, mmol/L	4.6 (4.4, 4.8)	4.3 (4.1,6.1)	0.737
F BGL, mg/dL	83 (79, 86)	77 (74, 110)	
2-h oGTT, mmol/L	5.9 (5.2,6.7)	11.2 (8.1,13.8)	0.009
2-h oGTT, mg/dL	106 (94, 121)	202 (146, 284)	
oGTT AUC	828 (761,908)	1448 (1336,1560)	0.030
HbA1c, %	5.2 (4.9,5.6)	4.9 (4.8,5.0)	0.348
HbA1c, mmol/mol	35.3 (30.1,37.7)	30.1 (29,31.1)	
HOMA2IR	1.0 (0.8, 1.4)	1.3 (1.1,2.0)	0.218
FPIR	25.4 (19.8, 33.7)	29.8 (15.8, 51.8)	0.773
DI (IV)	23.3 (19.4,27.9)	25.9 (14.4,31.6)	1.000

All values expressed as median (25%,75% percentile) unless specified.
Dysglycemia is defined by 2 hour oGTT result of ≥7.8 mmol/L (140 mg/dl).

TABLE 3.

Recipient results at 12 months posttransplantation

Variable at 12 mo	Subgroup	Normoglycemic at 12 mo (N = 7)	Dysglycemic at 12 mo (N = 7)	P
Age		35 (32, 46)	51 (39, 59)	0.096
Calcineurin inhibitor	Tacrolimus	6 (85.7%)	3 (42.9%)	0.133
	Cyclosporine	1 (14.3%)	4 (57.1%)	
Prior rejection		3 (42.9%)	1 (14.3%)	0.280
Creatinine at 12 mo, µmol/L		119 (92, 141)	113 (112, 127)	0.360
Creatinine at 12 mo, mg/dL		1.3 (1.0, 1.6)	1.3 (1.3, 1.4)	
F BGL, mmol/L		5.0 (4.5, 5.2)	6.0 (4.9, 6.8)	0.084
F BGL, mg/dl		90 (81, 94)	108 (88, 122)	
2-h oGTT glucose, mmol/L		5.7 (4.7, 6.5)	10.0 (9.2, 11.2)	0.002
2-h oGTT glucose, mg/dL		103 (85, 117)	180 (166, 202)	
oGTT AUC		863 (785, 901)	1198 (1099, 1351)	0.006
HbA1c %		5.3 (5.2, 5.5)	5.6 (5.5, 6.2)	0.040
HbA1c, mmol/mol		34.4 (33.3,36.6)	37.7 (36.6,44.3)	
HOMA2IR		1.2 (1.0, 1.5)	1.4 (1.2, 2.9)	0.198
FPIR		31.1 (25.3, 34.9)	17.2 (3.2, 32.7)	0.159
DI (IV)		23.0 (21.0, 34.6)	12.2 (3.2, 18.3)	0.007

All values expressed as median (25%,75% percentile) unless specified.
Dysglycemia is defined by 2 hour oGTT result of ≥7.8 mmol/L (140 mg/dl).

We then analyzed recipient data at 3 months posttransplantation stratified according to 12 month glycemic status (Table 4A). At 3 months posttransplantation those who were classified as dysglycemic at 12 months had a higher 2 hour

TABLE 4A.

Recipient results at 3 months stratified by 12 month glycemic state

Variable at 3 mo	Normoglycemic at 12 mo (N = 7)	Dysglycemic at 12 mo (N = 7)	P
Tacrolimus exposure (AUC for 3 mo, n = 9)	90 (87, 96)	95 (82, 109)	0.696
Cyclosporine Exposure (AUC for 3 mo, n = 5)	10544 (N = 1)	13529 (12773, 14921)	N/A
Median Creatinine over 3 mo, µmol/L	121 (90, 131)	137 (114, 153)	0.142
Median creatinine over 3 mo, mg/dL	1.4 (1.0, 1.5)	1.5 (1.3, 1.7)	
F BGL, mmol/L	5.2 (4.8, 6.0)	5.5 (4.8, 8.2)	0.443
F BGL, mg/dL	94 (86, 108)	99 (86, 148)	
2-h oGTT, mmol/L	6.8 (5.1, 8.0)	12.1 (9.1, 16.1)	0.009
2-h oGTT, mg/dL	122 (92, 144)	218 (164, 290)	
oGTT AUC	897 (773, 1034)	1411 (1047, 1689)	0.009
HbA1c%	5.4 (4.7, 5.6)	6.2 (5.4, 8.4)	0.098
HbA1c, mmol/mol	35.5 (27.9,37.7)	44.3 (35.5,68.3)	
HOMA2IR	1.3 (1.1, 1.4)	1.7 (0.9, 2.0)	0.564
FPIR	32.4 (28.9, 38.8)	7.8 (1.9, 48.0)	0.180
DI (IV)	27.0 (21.6, 35.1)	6.4 (1.4, 22.9)	0.025

All values expressed as median (25%,75% percentile) unless specified.
Dysglycemia is defined by 2 hour oGTT result of ≥7.8 mmol/L (140 mg/dl).

TABLE 4B.

Recipient results at day 7 stratified by 12-month glycemic state

Variable at day 7	Normoglycemic at 12 mo (N = 7)	Dysglycemic at 12 mo (N = 7)	P
F BGL, mmol/L	5.7 (5.1, 5.8)	5.0 (4.7, 5.8)	0.198
F BGL, mg/dL	103 (92, 104)	90 (85, 104)	
DI (IV)	14.4 (11.1, 30.6)	12.6 (10.2, 39.6)	1.000

oGTT (*P* = 0.035) and oGTT AUC (*P* = 0.0009). Significant separation (*P* = 0.018) of the glycemic response was evident at 60 minutes after an oral glucose load (Figure 3). Neither the 3-month F BGL nor HbA1c were discriminatory, and there were no significant differences in HOMA2IR. However, a striking decline in 3 month DI (IV) (27 vs 6.4, *P* = 0.025) was observed in those dysglycemic at 12 months. As depicted in Figure 4, a fall in DI (IV) by 3 months predicted dysglycemia at 12 months whereas a preserved DI (IV) at 3 months predicted normoglycemia at 12 months. Thus, recipients with similar CNI exposure and graft function at 3 months who are deemed dysglycemic at 12 months could be distinguished at 3 months from those normoglycemic at 12 months by features indicative of β cell dysfunction. We note that glucose handling was dynamic in 4 recipients: 2 recipients who were normoglycemic at 3 months subsequently became dysglycemic by 12 months, whereas 2 recipients who were dysglycemic at 3 months became normoglycemic at 12 months. Of the former group one recipient had a low DI (IV) (6.35) whereas the other had a preserved DI (IV) (28.3) at 3 months. The recipients who reverted to normoglycemia at 12 months both had preserved DI (IV) at 3 months (31.73 and 21.56).

β cell responsiveness changed quickly and we observed marked variance in DI (IV) in individuals at day 7 posttransplant, presumably reflecting surgical stress, high dose immunosuppression and dramatic changes in renal physiology. No recipients were receiving treatment for hyperglycemia at the time testing. We did not detect any correlation with the results at day 7 and the eventual development of dysglycemia. Furthermore, F BGL remained normal and did not distinguish between the 12 month glycemic states (Table 4B).

To identify individuals pretransplant at risk persistent of posttransplant dysglycemia we stratified recipients according to 3 month metabolic state and analyzed the data at study
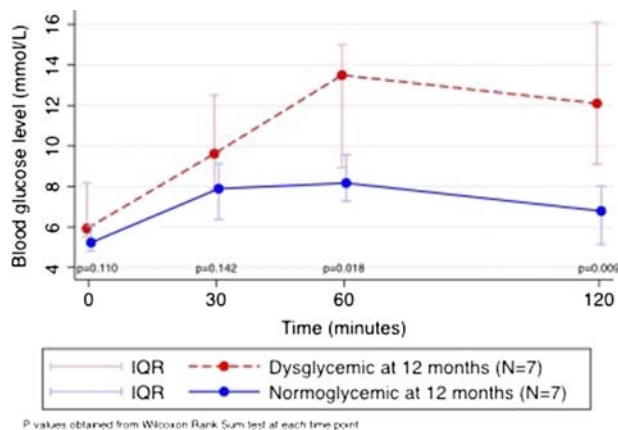

P values obtained from Wilcoxon Rank Sum test at each time point.

FIGURE 3. Oral glucose tolerance at 12 months.

P values obtained from Wilcoxon Rank Sum test at each time point.
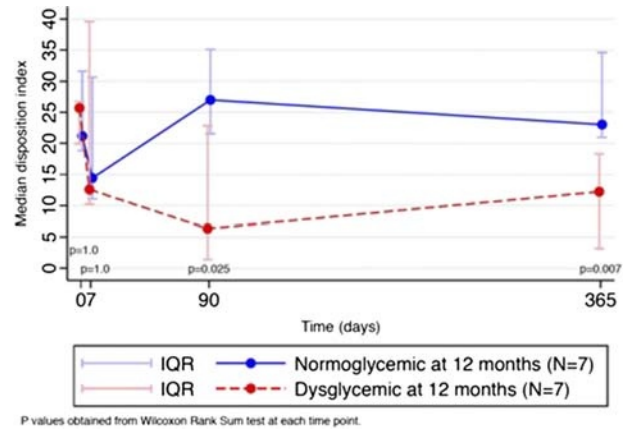
FIGURE 4. Disposition index over 12 months.

entry (Table 5). Three months posttransplantation was chosen as the underpinning pathophysiological changes accounting for 12 month dysglycemia, as measured by DI (IV), were evident by 3 months. We note the median 2 hour oGTT result was higher in those who progressed to dysglycemia at 3 months posttransplantation compared to those who remained normoglycemic (6.89 mmol/L vs 5.56 mmol/L (124 mg/dl vs 100 mg/dl), *P* = 0.01). The HbA1c was also higher in those that progressed to dysglycemia. Notably, although elevated compared to those who remained normoglycemic, neither parameter fulfilled the diagnostic criteria for diabetes in the general population. Although the FPIR, DI (IV) and HOMA2IR at study entry were similar between groups, these results suggest subtle baseline β cell abnormalities, which are exaggerated and made clinically apparent by the physiological stress of transplantation.

TABLE 5.

Recipient results at entry into study according to 3 month glycemic status

Variable at study entry	Normoglycemic at 3 mo (N = 9)	Dysglycemic at 3 mo (N = 7)	P
Age	39 (34, 49)	50 (32, 59)	0.314
Waist-hip ratio	.89 (.86, .99)	1.05 (0.87, 1.08)	0.153
Body mass index, kg/m²	25.5 (21.8, 29.9)	29.7 (25.4, 30.1)	0.315
Systolic blood pressure, mm Hg	130 (120, 150)	130 (115, 150)	0.831
Diastolic blood pressure, mm Hg	80 (80, 93)	80 (60, 90)	0.307
Total cholesterol, mmol/L	5.4 (4.4, 6.2)	4.2 (3.8, 5.0)	0.089
Total cholesterol, mg/dL	209 (170, 239)	162 (147, 193)1	
Triglycerides, mmol/L	1.3 (1.2, 1.6)	2.1 (1.6, 2.7)	0.138
Triglycerides, mg/dL	115 (106, 142)	186 (142, 239)	
F BGL, mmol/L	4.6 (4.4, 4.8)	4.5 (4.1, 5.0)	0.491
F BGL, mg/dL	83 (79, 86)	81 (74, 90)	
2-h oGTT, mmol/L	5.6 (4.8, 6.2)	6.8 (6.7, 8.1)	0.010
2-h oGTT, mg/dL	101 (86, 112)	122 (121, 146)	
oGTT AUC	828 (788, 901)	1921 (834.5, 1247)	0.280
HbA1c%	4.9 (4.7, 5.1)	5.3 (5.2, 5.6)	0.051
HbA1c, mmol/mol	30.1 (27.9, 32.2)	34.4 (33.3, 37.7)	
HOMA2IR	1.0 (0.9, 1.4)	1.2 (0.8, 1.4)	0.768
FPIR	28.7 (18.8, 36.7)	25.5 (20.7, 30.7)	0.906
DI (IV)	25.5 (20.0, 28.7)	23.5 (18.8, 26.7)	0.906

All values expressed as median (25%,75% percentile) unless specified.
Dysglycemia is defined by 2 hour oGTT result of ≥7.8 mmol/L (140 mg/dL).

DISCUSSION

The development of dysglycemia post renal transplantation is highly prevalent and confers an increased risk of cardiovascular disease that manifests rapidly.[1-5] There is, however, a paucity of literature documenting the dynamic changes and underlying pathophysiological mechanisms occurring in glucose metabolism that accompany transplantation. The stress of surgery, exposure to multiple diabetogenic medications and improved renal function are all likely to alter glucose metabolism. We analyzed longitudinally over the course of a year the glucometabolic parameters of renal transplant recipients. Importantly, our data suggests that a major contributor to dysglycemia after renal transplantation is impaired insulin secretion due to progressive reduction in β-cell responsiveness. In addition, our findings highlight that recipients at risk for posttransplant dysglycemia may have subtle abnormalities of glucose handling evident on pretransplant oGTT and these recipients may be further stratified at 3 months with reference to tests of the underlying pathophysiology.

We compared our recipient cohort with a group of potential living donors who are typical of a healthy general population. No differences could be found pretransplant between either of these groups on the basis of routine glucose handling tests, such as F BGL and HbA1c. Within the recipient cohort there were 3 cases of unrecognized dysglycemia, diagnosed only by oGTT. As we observed, and consistent with others,[8-10] pretransplant dysglycemia was not necessarily associated with posttransplant dysglycemia highlighting the important contribution of renal function to insulin sensitivity and glucose handling. In patients with end stage renal failure relative insulin resistance may manifest with fasting hyperglycemia and abnormal glucose responses during oGTT and IV GTT, whereas others maintain normoglycemia with hyperinsulinemia.[26] These findings highlight the difficulty that transplant clinicians encounter when attempting to assess risk for posttransplant dysglycemia using standard clinical investigations.

We defined the glycemic state posttransplantation as dysglycemic or normoglycemic because the clinical ramifications of cardiovascular mortality accrue with either IGT or PTDM.[1-5] Furthermore, by defining glycemic state with reference to 12 month data we excluded transient dysglycemia as recommended by the International Consensus on Posttransplant Diabetes.[6] Consistent with others,[10,14] we noted changes in definitional states (IGT vs PTDM) over the first year; however, found stability in the more granular definition of persistent dysglycemia.

Our hypothesis was that the underlying mechanism of persistent dysglycemia posttransplantation was a reduction in β cell responsiveness. Reduced FPIR to a glucose bolus has been recognized as a critical factor in the genesis of dysglycemia in the general population particularly in the presence of ongoing insulin resistance.[20] In transplantation, some have described an increase in insulin resistance as the predominant cause for impairment in glucose metabolism posttransplantation,[10] others have emphasized the importance of reduced insulin secretion.[5,15,27,28] Our results build on these previous findings by prospectively studying the changes in glucometabolic physiology pre, early and late posttransplantation. In our recipients, insulin resistance was comparable between normoglycemic and dysglycemic groups throughout the 12 month period. Dysglycemia was however

associated with a fall in DI (IV), which in light of dysglycemic recipient insulin resistance being comparable to our comparator group (1.4 vs 1.2), reflects a loss of β cell responsiveness. consistent with a marked decline in β cell responsiveness as the primary lesion for the dysglycemic state.

Our findings also suggest that it is possible to detect at 3 months those who will be dysglycemic at 12 months. The fall in DI was evident early posttransplantation making it possible to detect at 3 months those who will be dysglycemic at 12 months. Such findings are supported by other published results; for example, the findings of Porrini et al[29] suggest that recipients who are normoglycemic at 3 months on oGTT testing rarely develop diabetes later posttransplantation. We add to this finding firstly by investigating all dysglycemic states and not diabetes mellitus exclusively. By doing so, we show that those who are dysglycemic at 12 months are significantly likely to have higher 2 hour oGTT results at 3 months. Furthermore, the 12 month dysglycemic patients have significantly higher 1 hour results on 3 month oGTT. These findings are consistent with those in the general population when attempting to identify those at highest risk of the development of type 2 diabetes mellitus[30] and highlight reduced β cell function as the primary lesion in persistent (12 month) dysglycemia. Together these findings suggest that early posttransplant is an opportune time for the implementation of interventions that may potentially preserve insulin secretion. Such a strategy has been implemented by Hecking et al[17] who showed that a reduction in PTDM could be achieved through the early administration of insulin.

Ideally, clinicians would have reliable tests that could detect pre, or very early posttransplant those recipients at greatest risk of dysglycemia. Some groups have reported that different clinical tests of glucose handling within the first week posttransplantation may be a strong predictor for the development of posttransplant diabetes.[31-35] We found that tests of glucose handling at this time point have low discriminative value for later dysglycemia. Indeed, only one recipient had an abnormal F BGL at day 7 (6.91 mmol/L (124 mg/dl)) and this recipient did not have evidence of dysglycemia pretransplant, or at 3 and 12 months posttransplant. Conversely all recipients who later developed dysglycemia had a normal fasting BGL at day 7.

Although early posttransplant testing was not discriminatory, pretransplant testing did aid in detecting those dysglycemic at 3 months. Using 2 hour oGTT testing, we detected significant differences in glucose handling pretransplant in those who progressed to dysglycemia at 3 months with an associated decline in DI (IV). Interestingly the 2 hour BGL remained within normal limits for healthy individuals but was significantly greater than in those individuals who did not progress to dysglycemia. Others have described similar data: Nam et al used oGTT pretransplant and at 9 to 12 months posttransplant and found that recipients with higher, albeit normal, pretransplant 2 hour oGTT were significantly more likely to have posttransplant dysglycemia.[28] A retrospective analysis of 145 recipients who underwent a pretransplant oGTT (but without posttransplant oGTT) found that the 5 time point 2 hour oGTT AUC identified those recipients at highest risk for PTDM. Once more, consistent with findings in the general population, the authors also identified that nondiabetic patients with higher 1 hour oGTT had increased risk of PTDM.[36] Although we are limited by our small sample size,

our similar findings may indicate subclinical pretransplant β cell susceptibility, which fails under the stressors of transplantation. Despite different groups demonstrating potential susceptibility by oGTT testing, we and others[5] have not been able to identify pretransplant pathophysiological changes that may account for this susceptibility.

The above findings suggest that the pretransplant thresholds for at risk individuals may differ from the general population. Indeed, Rosettenstein et al[37] used the combination of fructosamine and lower HbA1c thresholds to identify at risk patients who proceed to formal oGTT. Yates et al[38] have shown that lower HbA1c thresholds assist in detection of at risk individuals by increasing negative predictive values. It should be noted however that HbA1c results are limited by anemia, which can be found posttransplant, and oGTT remains the gold standard tests. We suggest that altered physiology associated with ESKD pretransplant and CKD posttransplant may warrant changes in the diagnostic values that define clinically important glycemic states, similar to the example of gestational diabetes.[39]

Transplant specific immunosuppressive medications alter glucose handling: Glucocorticoids increase insulin resistance[15,40] and CNI use has been associated with decreased insulin secretion.[5,28,41-43] In this study all patients had ongoing, albeit reducing, exposure to glucocorticoids. Although a modest increase in median weight was documented over 12 months, we did not detect any clinically relevant change in insulin resistance. Our cohort contained patients treated with both tacrolimus and cyclosporine; however, the small sample size precluded comparisons between the groups. A higher incidence of PTDM[15,44-46] and greater reduction in insulin secretion has been reported in patients treated with tacrolimus compared to cyclosporin.[15] Consistent with this effect our data implicated a reduction in insulin secretion as a major contributor to dysglycemia.

The strengths of our study are that it is the first prospective study using intravenous and oGTT of renal transplant recipients. As the pretransplant test was conducted within 2 months of transplantation, there was minimal risk of change in the glycemic state pretransplant and we were able to compare the recipients with healthy kidney donors. The combination of intravenous and oral glucose testing enabled a descriptive longitudinal analysis of β cell responsiveness in relation to changing insulin resistance in individual recipients to be documented. We have also accounted for the CNI exposure over the first 3 months in a more comprehensive manner than by reference to drug level measurements alone. The main limitation of this study is that the recipient numbers are small limiting the ability to analyze the interaction between variables and indeed may account for the lack of significance of clinically relevant investigations, such as BMI and HbA1c. Our results are drawn from a single transplant consortium that while used a standard and protocolized triple therapy immunosuppression regimen allowed for choice between CNI agents and may not be generalizable to all transplant populations. The small numbers limits our ability to draw conclusions with regard to the alternative CNI agents. This was not a study designed to distinguish the diabetogenic effects of specific immunosuppression agents we did not withhold agents in the period immediately before testing; however, this zis only a relative limitation as this reflects actual clinical practice. Furthermore, although there is some evidence of

glycemic benefit through the use of DPP-4 inhibitors[47,48] we did not address the role of incretins in PTDM. There is data in the general healthy population and relatives of Type 2 diabetics that glucocorticoid exposure may impair the incretin effect.[49] There is also some data on the impairment of incretins in dialysis recipients,[50] however, to date there is no data on the incretin effect in the renal transplant population.

In conclusion, we have shown that posttransplant dysglycemia is common and difficult to recognize without oGTT testing. In addition, standard pretransplant clinical assessment alone is not sufficient for identifying those at high risk of developing dysglycemia. Importantly a subclinical β-cell deficiency, which is unmasked by transplant related factors, may be detected by pretransplant oGTT. In addition, rigorous testing at 3 months may reliably predict permanent dysglycemia. We have shown that intravenous glucose tolerance testing can be used in renal transplant recipients and the indices derived from such tests (in particular DI (IV)) demonstrate that a loss of β-cell responsiveness is a major contributing factor to dysglycemia posttransplant. Validation of these results in larger cohorts would enable the reliable identification of higher risk patients (potentially pretransplant and/or at 3 months), which then permits the implementation of strategies to reduce permanent dysglycemia. Such strategies may include closer therapeutic drug monitoring, the utilization of alternative immunosuppressive regimens or planned and prophylactic posttransplant insulin administration.[17]

REFERENCES

1. Cosio FG, Kudva Y, van der Velde M, et al. New onset hyperglycemia and diabetes are associated with increased cardiovascular risk after kidney transplantation. *Kidney Int*. 2005;67:2415–2421.
2. Hjelmesaeth J, Hartmann A, Leivestad T, et al. The impact of early-diagnosed new-onset post-transplantation diabetes mellitus on survival and major cardiac events. *Kidney Int*. 2006;69:588–595.
3. Valderhaug TG, Hjelmesæth J, Hartmann A, et al. The association of early post-transplant glucose levels with long-term mortality. *Diabetologia*. 2011;54:1341–1349.
4. Wauters RP, Cosio FG, Suarez Fernandez ML, et al. Cardiovascular consequences of new-onset hyperglycemia after kidney transplantation. *Transplantation*. 2012;94:377–382.
5. Nagaraja P, Ravindran V, Morris-Stiff G, et al. Role of insulin resistance indices in predicting new-onset diabetes after kidney transplantation. *Transpl Int*. 2013;26:273–280.
6. Sharif A, Hecking M, de Vries AP, et al. Proceedings From an International Consensus Meeting on Posttransplantation Diabetes Mellitus: Recommendations and Future Directions. *Am J Transplant*. 2014;14: 1992–2000.
7. Bergrem HA, Valderhaug TG, Hartmann A, et al. Undiagnosed diabetes in kidney transplant candidates: a case-finding strategy. *Clin J Am Soc Nephrol*. 2010;5:616–622.
8. Caillard S, Eprinchard L, Perrin P, et al. Incidence and risk factors of glucose metabolism disorders in kidney transplant recipients: Role of systematic screening by oral glucose tolerance test. *Transplantation*. 2011;91: 757–764.
9. Iida S, Ishida H, Tokumoto T, et al. New-onset diabetes after transplantation in tacrolimus-treated, living kidney transplantation: long-term impact and utility of the pre-transplant OGTT. *Int Urol Nephrol*. 2010;42:935–945.
10. Hornum M, Jørgensen KA, Hansen JM, et al. New-onset diabetes mellitus after kidney transplantation in Denmark. *Clin J Am Soc Nephrol*. 2010;5: 709–716.
11. Werzowa J, Hecking M, Haidinger M, et al. The diagnosis of posttransplantation diabetes mellitus: meeting the challenges. *Curr Diab Rep*. 2015;15:27.
12. Bergrem HA, Valderhaug TG, Hartmann A, et al. Glucose tolerance before and after renal transplantation. *Nephrol Dial Transplant*. 2010;25: 985–992.

13. Chakkera HA, Pham PT, Pomeroy J, et al. Response to Comment on: Chakkera et al. Can new-onset diabetes after kidney transplant be prevented? Diabetes Care 2013;36:1406-1412. *Diabetes Care*. 2013; 36:e183.

14. Chan L, Andres A, Bunnapradist S, et al. Renal function and NODM in de novo renal transplant recipients treated with standard and reduced levels of tacrolimus in combination with EC-MPS. *J Transplant*. 2012; 2012:941640.

15. Vincenti F, Friman S, Scheuermann E, et al. Results of an international, randomized trial comparing glucose metabolism disorders and outcome with cyclosporine versus tacrolimus. *Am J Transplant*. 2007;7:1506–1514.

16. Montero N, Pascual J. Immunosuppression and post-transplant hyperglycemia. *Curr Diabetes Rev*. 2015;11:144–154.

17. Hecking M, Haidinger M, Döller D, et al. Early basal insulin therapy decreases new-onset diabetes after renal transplantation. *J Am Soc Nephrol*. 2012;23:739–749.

18. Bergman RN, Ader M, Huecking K, et al. Accurate assessment of beta-cell function: the hyperbolic correction. *Diabetes*. 2002;51(Suppl 1): S212–S220.

19. Lorenzo C, Wagenknecht LE, Rewers MJ, et al. Disposition index, glucose effectiveness, and conversion to type 2 diabetes: the insulin resistance atherosclerosis study (IRAS). *Diabetes Care*. 2010;33:2098–2103.

20. Utzschneider KM, Prigeon RL, Faulenbach MV, et al. Oral disposition index predicts the development of future diabetes above and beyond fasting and 2-h glucose levels. *Diabetes Care*. 2009;32:335–341.

21. Alford FP, Rantzau C, Henriksen JE, et al. Endogenous incretin hormone augmentation of acute insulin secretion in normoglycemic relatives of type 2 diabetic subjects: a 10-year retrospective pathophysiological study. *J Clin Endocrinol Metab*. 2014;99:E1943–E1950.

22. Marini MA, Succurro E, Frontoni S, et al. Insulin sensitivity, β-cell function, and incretin effect in individuals with elevated 1-hour postload plasma glucose levels. *Diabetes Care*. 2012;35:868–872.

23. Pacini G, Bergman RN. MINMOD: a computer program to calculate insulin sensitivity and pancreatic responsivity from the frequently sampled intravenous glucose tolerance test. *Comput Methods Programs Biomed*. 1986;23:113–122.

24. Niemczyk S, Szamotulska K, Giers K, et al. Homeostatic model assessment indices in evaluation of insulin resistance and secretion in hemodialysis patients. *Med Sci Monit*. 2013;19:592–598.

25. Defronzo RA, Tripathy D, Schwenke DC, et al. Prediction of diabetes based on baseline metabolic characteristics in individuals at high risk. *Diabetes Care*. 2013;36:3607–3612.

26. Mak RH. Impact of end-stage renal disease and dialysis on glycemic control. *Semin Dial*. 2000;13:4–8.

27. Hecking M, Kainz A, Werzowa J, et al. Glucose metabolism after renal transplantation. *Diabetes Care*. 2013;36:2763–2771.

28. Nam JH, Mun JI, Kim SI, et al. beta-Cell dysfunction rather than insulin resistance is the main contributing factor for the development of postrenal transplantation diabetes mellitus. *Transplantation*. 2001;71:1417–1423.

29. Porrini EL, Díaz JM, Moreso F, et al. Clinical evolution of post-transplant diabetes mellitus. *Nephrol Dial Transplant*. 2016;31:495–505.

30. Alyass A, Almgren P, Akerlund M, et al. Modelling of OGTT curve identifies 1 h plasma glucose level as a strong predictor of incident type 2 diabetes: results from two prospective cohorts. *Diabetologia*. 2015;58:87–97.

31. Kuypers DR, Claes K, Bammens B, et al. Early clinical assessment of glucose metabolism in renal allograft recipients: diagnosis and prediction of post-transplant diabetes mellitus (PTDM). *Nephrol Dial Transplant*. 2008; 23:2033–2042.

32. Rodrigo E, Santos L, Piñera C, et al. Early prediction of new-onset diabetes mellitus by fifth-day fasting plasma glucose, pulse pressure, and proteinuria. *Transplant Proc*. 2011;43:2208–2210.

33. Joss N, Staatz CE, Thomson AH, et al. Predictors of new onset diabetes after renal transplantation. *Clin Transplant*. 2007;21:136–143.

34. Wojtusciszyn A, Mourad G, Bringer J, et al. Continuous glucose monitoring after kidney transplantation in non-diabetic patients: early hyperglycaemia is frequent and may herald post-transplantation diabetes mellitus and graft failure. *Diabetes Metab*. 2013;39:404–410.

35. Chakkera HA, Knowler WC, Devarapalli Y, et al. Relationship between inpatient hyperglycemia and insulin treatment after kidney transplantation and future new onset diabetes mellitus. *Clin J Am Soc Nephrol*. 2010;5: 1669–1675.

36. Tokodai K, Amada N, Haga I, et al. The 5-time point oral glucose tolerance test as a predictor of new-onset diabetes after kidney transplantation. *Diabetes Res Clin Pract*. 2014;103:298–303.

37. Rosettenstein K, Viecelli A, Yong K, et al. Diagnostic accuracies of glycated hemoglobin, fructosamine, and homeostasis model assessment of insulin resistance in predicting impaired fasting glucose, impaired glucose tolerance, or new onset diabetes after transplantation. *Transplantation*. 2016;100:1571–1579.

38. Yates CJ, Fourlanos S, Colman PG, et al. Screening for new-onset diabetes after kidney transplantation: limitations of fasting glucose and advantages of afternoon glucose and glycated hemoglobin. *Transplantation*. 2013;96:726–731.

39. World Health Organization. Diagnostic Criteria and Classification of Hyperglycaemia First Detected in Pregnancy. 2013 available at http://apps.who.int/iris/bitstream/10665/85975/1/WHO_NMH_MND_13.2_eng.pdf

40. Hwang JL, Weiss RE. Steroid-induced diabetes: a clinical and molecular approach to understanding and treatment. *Diabetes Metab Res Rev*. 2014;30:96–102.

41. Zelle DM, Corpeleijn E, Deinum J, et al. Pancreatic β-cell dysfunction and risk of new-onset diabetes after kidney transplantation. *Diabetes Care*. 2013;36:1926–1932.

42. Hjelmesaeth J, Hagen M, Hartmann A, et al. The impact of impaired insulin release and insulin resistance on glucose intolerance after renal transplantation. *Clin Transplant*. 2002;16:389–396.

43. Hagen M, Hjelmesaeth J, Jenssen T, et al. A 6-year prospective study on new onset diabetes mellitus, insulin release and insulin sensitivity in renal transplant recipients. *Nephrol Dial Transplant*. 2003;18: 2154–2159.

44. Ekberg H, Bernasconi C, Nöldeke J, et al. Cyclosporine, tacrolimus and sirolimus retain their distinct toxicity profiles despite low doses in the symphony study. *Nephrol Dial Transplant*. 2010;25:2004–2010.

45. Cole EH, Johnston O, Rose CL, et al. Impact of acute rejection and new-onset diabetes on long-term transplant graft and patient survival. *Clin J Am Soc Nephrol*. 2008;3:814–821.

46. Luan FL, Steffick DE, Ojo AO. New-onset diabetes mellitus in kidney transplant recipients discharged on steroid-free immunosuppression. *Transplantation*. 2011;91:334–341.

47. Haidinger M, Werzowa J, Hecking M, et al. Efficacy and safety of vildagliptin in new-onset diabetes after kidney transplantation—a randomized, double-blind, placebo-controlled trial. *Am J Transplant*. 2014; 14:115–123.

48. Haidinger M, Antlanger M, Kopecky C, et al. Post-transplantation diabetes mellitus: evaluation of treatment strategies. *Clin Transplant*. 2015;29: 415–424.

49. Hansen KB, Vilsbøll T, Bagger JI, et al. Impaired incretin-induced amplification of insulin secretion after glucose homeostatic dysregulation in healthy subjects. *J Clin Endocrinol Metab*. 2012;97:1363–1370. doi:10. 1210/jc.2011-2594.

50. Idorn T, Knop FK, Jørgensen M, et al. Postprandial responses of incretin and pancreatic hormones in non-diabetic patients with end-stage renal disease. *Nephrol Dial Transplant*. 2014;29:119–127.