Pancreatic β-Cell Dysfunction and Risk of New-Onset Diabetes After Kidney Transplantation

Dorien M. Zelle, msc[1]
Eva Corpeleijn, phd[1,2]
Jolijn Deinum, msc[1]
Ronald P. Stolk, md, phd[2]
Rijk O.B. Gans, md, phd[3]
Gerjan Navis, md, phd[1]
Stephan J.L. Bakker, md, phd[3]

OBJECTIVE—Chronic exposure to calcineurin inhibitors and corticosteroids poses renal transplant recipients (RTR) at high risk for development of new-onset diabetes after transplantation (NODAT). Pancreatic β-cell dysfunction may be crucial to the pathophysiology of NODAT and specific markers for β-cell dysfunction may have additive value for predicting NODAT in this population. Therefore, we prospectively investigated whether proinsulin, as a marker of pancreatic β-cell dysfunction, is associated with future development of NODAT and improves prediction of it.

RESEARCH DESIGN AND METHODS—All RTR between 2001 and 2003 with a functioning graft for ≥1 year were considered eligible for inclusion, except for subjects with diabetes at baseline who were excluded. We recorded incidence of NODAT until April 2012.

RESULTS—A total of 487 RTR (age 50 ± 12 years, 55% men) participated at a median time of 6.0 (interquartile range [IQR], 2.6–11.5) years after transplantation. Median fasting proinsulin levels were 16.6 (IQR, 11.0–24.2) pmol/L. During median follow-up for 10.1 (IQR, 9.1–10.4) years, 42 (35%) RTR had development of NODAT in the highest quartile of the distribution of proinsulin versus 34 (9%) in the lowest three quartiles ($P < 0.001$). In Cox regression analyses, proinsulin (hazard ratio, 2.29; 95% CI, 1.85–2.83; $P < 0.001$) was strongly associated with NODAT development. This was independent of age, sex, calcineurine inhibitors, prednisolone use, components of the metabolic syndrome, or homeostasis model assessment.

CONCLUSIONS—In conclusion, fasting proinsulin is strongly associated with NODAT development in RTR. Our results highlight the role of β-cell dysfunction in the pathophysiology of NODAT and indicate the potential value of proinsulin for identification of RTR at increased risk for NODAT.

Diabetes Care 36:1926–1932, 2013

New-onset diabetes after transplantation (NODAT) is one of the main metabolic complications of renal transplantation (1). It is estimated to affect ~20% of renal transplant recipients (RTR) (2). NODAT places RTR at an increased risk for infections, cardiovascular disease, graft failure, and mortality (2–4). Comparable with type 2 diabetes, NODAT may be a result of increased insulin resistance and decreased insulin production by the pancreatic β-cell (5). Early identification of increased risk for NODAT, allowing for early intervention, could be of great importance to renal transplant health care considering the detrimental effects associated with NODAT.

The presence of pretransplantation insulin resistance in the final stage of kidney failure is seen as a mechanism in the development of NODAT (6). The chronic exposure to calcineurin inhibitors and corticosteroids aggravates the insulin resistance and poses RTR at high risk for NODAT development (7,8). Another potential mechanism in NODAT is a defect in insulin secretion as a consequence of pancreatic β-cell dysfunction, leading to an inability to compensate for insulin resistance (5,6).

As a precursor of insulin, intact proinsulin has been proposed as a specific marker of β-cell dysfunction (5). In the past, nonspecific assays showed high cross-reactivity that could lead to incorrect conclusions on β-cell dysfunction and prediction of diabetes. A new, specific, intact proinsulin ELISA (no cross-reactivity) has been developed that can be easily used in routine laboratories (9).

It is unknown whether proinsulin is a good marker of β-cell dysfunction in RTR and whether it is independently associated with future development of NODAT or if it predicts NODAT beyond established clinical risk predictors. Therefore, we prospectively investigated the association between β-cell dysfunction, insulin resistance, and NODAT development in RTR. Furthermore, we investigated whether proinsulin had additive value in the prediction of NODAT.

RESEARCH DESIGN AND METHODS

Design and subjects

Study design and inclusion/exclusion criteria have been described previously (10). In brief, for this prospective cohort study all adult allograft recipients between August 2001 and July 2003 who survived with a functioning allograft beyond the first year after transplantation were eligible to participate at their next visit to our outpatient clinic. A total of 606 from an eligible 847 RTR (72% consent rate) signed written informed consent. We excluded 105 recipients with existing diabetes (defined as fasting plasma glucose ≥7.0 or antidiabetic medication) at baseline from analysis. Proinsulin levels were available in 487 RTR, leaving 487 nondiabetic RTR for analysis. Baseline data were collected between August 2001 and July 2003, and RTR were followed-up for several years. The Institutional Review Board approved the study protocol (METc 2001/039).

From the [1]Department of Nephrology, University Medical Center Groningen, Groningen, the Netherlands; the [2]Department of Epidemiology, University Medical Center Groningen, Groningen, the Netherlands; and the [3]Department of Internal Medicine, University Medical Center Groningen, Groningen, the Netherlands.
Corresponding author: Dorien M. Zelle, d.m.zelle@umcg.nl.
Received 17 September 2012 and accepted 27 December 2012.
DOI: 10.2337/dc12-1894

Renal transplant characteristics

The Groningen Renal Transplant Database contains information on all renal transplantations performed at our center since 1968. Relevant transplant recipient characteristics such as age, sex, and date of transplantation were extracted from this database. We found current medication information in the medical record and obtained information on employment status, living situation, smoking and alcohol consumption, and cardiovascular history by self-report questionnaire.

Standard immunosuppressive treatment consisted of the following: prednisolone and azathioprine (100 mg/day) from 1968 to 1989; cyclosporine standard formulation (trough levels of 175 to 200 mg/L in the first 3 months, 150 mg/L between 3 and 12 months after transplantation, and 100 mg/L thereafter; Sandimmune; Novartis Pharma B.V., Arnhem, the Netherlands) and prednisolone (starting with 20 mg/day, rapidly tapered to 10 mg/day) from January 1989 to February 1993; cyclosporine microemulsion (trough levels idem; Neoral; Novartis Pharma B.V.) and prednisolone from March 1993 to May 1997; and mycophenolate mofetil (2 g/day; Cellcept; Roche B.V., Woerden, the Netherlands), which was added from May 1997 to present date. In some specific situations, immunosuppressive medication deviated from the standard protocol. Cyclosporine was converted to tacrolimus in the event of acute rejection, hypertrichosis, gingival hypertrophy, or intolerance of cyclosporine. Target trough levels of tacrolimus were 6 to 10 μg/L. Sirolimus was used when frequent skin malignancy occurred. Target trough levels of sirolimus were 4 to 6.5 μg/L.

Measurements and definitions

BMI was determined as a measure of overall obesity. BMI was calculated as weight in kilograms divided by height in meters squared. Waist circumference was measured on bare skin midway between the iliac crest and the 10th rib. Muscle mass was estimated by 24-h creatinine excretion as described previously (11). Blood pressure was measured after a 6-min rest in the supine position as the average of three automated measurements at 1-min intervals (Omron M4; Omron Europe B.V.).

Blood was drawn after an overnight fasting period. Plasma glucose, insulin, HDL cholesterol, LDL cholesterol, high-sensitivity C-reactive protein, and serum creatinine were measured as described previously (10). Homeostasis model assessment (HOMA) was calculated as: [glucose (mmol/L) × insulin (in microunits/mL)]/22.5 (12). In this study, metabolic syndrome (MS) was defined according to the definition of the National Cholesterol Education Program Expert Panel (NCEP-ATPIII) (13).

Proinsulin. Proinsulin levels were measured with the Mercodia-Proinsulin ELISA. Mercodia-Proinsulin ELISA is a solid phase, two-site enzyme immunoassay based on the sandwich technique, in which two monoclonal antibodies are directed against separate antigenic determinants on the proinsulin molecule. Proinsulin in the sample reacts with anti-proinsulin antibodies bound to microtitration wells and peroxidase-conjugated anti-insulin antibodies in the solution. Cross-reactivity for insulin is <0.03% and cross-reactivity for C-peptide is <0.006%.

NODAT. The International Expert Panel Meeting (14) proposed recommendations to define NODAT based on the American Diabetes Association criteria 2003 (15). The diagnosis of NODAT was based on one of the following criteria: symptoms of diabetes (classic symptoms of diabetes include polyuria, polydipsia, and unexplained weight loss) plus casual plasma glucose concentration ≥200 mg/dL (11.1 mmol/L); fasting plasma glucose ≥126 mg/dL (7.0 mmol/L); or use of antidiabetic medication. Fasting is defined as no caloric intake for at least 8 h. NODAT was recorded until April 2012.

Statistical analyses

Data were analyzed with SPSS version 16.0 (SPSS, Chicago, IL), STATA version 11 (StataCorp LP), and GraphPad Prism version 4.03 (GraphPad Software, San Diego, CA). Recipient-related characteristics were analyzed separately for quartiles of proinsulin. For analyses, we combined quartiles 1–3 as one group and compared this group with quartile 4. Differences between groups were tested for statistical significance with Student *t* test for normally distributed variables, Mann-Whitney test for skewed distributed variables, and χ^2 test for categorical variables. We performed multivariate Cox regression analyses to investigate whether proinsulin is independently associated with NODAT. In subsequent multivariate analyses, we investigated whether the association of proinsulin is independent of age, sex, use of cyclosporine, tacrolimus, dose of prednisolone, trough levels of cyclosporine and tacrolimus, HOMA, or components of the metabolic syndrome. Death was regarded as a competing risk for NODAT. Patients were censored at date of last follow-up or death.

Because there are no validated clinical models for the prediction of NODAT in RTR, we used a commonly used prediction model for the general population. This clinical model includes the following: sex, smoking, waist circumference, hypertension, and family history of diabetes (16). To assess the added value of proinsulin, we examined improvement of diabetes prediction in terms of discrimination and integrated discrimination improvement (IDI). Discrimination was evaluated using the Harrell c-index for censored data, a statistic similar to the area under a receiver-operating characteristic curve (17). In general, discrimination refers to the ability of a model to distinguish well between individuals with and without incident diabetes; a value of 1 implies a perfect discrimination and a value of 0.5 implies performance no better than chance. We used IDI as a continuous measure of reclassification, calculated by subtracting the mean difference of predicted risk between the clinical model (16) and the model including different biomarkers.

RESULTS—The study cohort was composed of 487 RTR (55% men) aged 50 ± 12 years at a median time of 6.0 (interquartile range, 2.6–11.5) years after transplantation. Median concentration of fasting proinsulin was 16.6 (interquartile range, 11.0–24.2) pmol/L. Baseline characteristics of the RTR according to the two groups of proinsulin are shown in Table 1. RTR with high proinsulin (quartile 4 versus quartiles 1–3) were more obese, with higher BMI and waist circumference. High proinsulin was positively associated with use of β-blocker, use of statin, history of BMI, high-sensitivity C-reactive protein, glucose, insulin, HOMA, and proinsulin-to-insulin ratio. RTR with high proinsulin had significantly lower creatinine clearance and higher serum creatinine. We found other differences in lipid-profile with lower HDL and LDL cholesterol and higher triglycerides in subjects with high proinsulin. There was a trend toward lower use of tacrolimus in subjects with high proinsulin. No differences were found in other components of immunosuppressive treatment. Out of the 487 RTR, 309

Table 1—*Recipient characteristics according to groups of proinsulin*

	Quartiles of proinsulin		
	1–3 (*N* = 366)	4 (*N* = 121)	*P*
General characteristics			
Age (years)	50.4 ± 12.5	51.1 ± 11.0	0.6
Male sex, *n* (%)	203 (55.5)	68 (56.2)	0.9
Lifestyle			
Physical activity (METS)	124.5 (31.2–312.1)	118.7 (26.7–294.1)	0.4
MS, *n* (%)	208 (57)	101 (86)	<0.001
Smoking, *n* (%)	150 (41.3)	48 (39.7)	0.8
Alcohol consumption			
Abstain, *n* (%)	160 (44)	63 (53)	
<10 g/day, *n* (%)	145 (40)	44 (37)	
10–30 g/day, *n* (%)	52 (14)	13 (11)	0.2
>30 g/day, *n* (%)	6 (2)	0 (0)	
Body composition			
BMI (kg/m^2)	25.1 ± 3.9	27.8 ± 4.2	<0.001
Waist circumference (cm), women	90.4 ± 13.3	98.8 ± 13.8	<0.001
Waist circumference (cm), men	96.6 ± 11.1	105.7 ± 10.9	<0.001
Urinary creatinine excretion (mmol/24 h)	11.9 (9.5–14.4)	11.9 (9.8–13.9)	0.9
Blood pressure			
Systolic pressure (mmHg)	152.0 ± 23.1	149.6 ± 20.9	0.3
Diastolic pressure (mmHg)	90.0 ± 10.1	89.3 ± 9.3	0.5
Use of ACE inhibitor or angiotensin II antagonist, *n* (%)	117 (32)	49 (40.5)	0.09
Use of β-blocker, *n* (%)	216 (59.0)	87 (71.9)	0.01
History of cardiovascular disease			
Myocardial infarction, *n* (%)	24 (6.6)	16 (13.2)	0.02
Transient ischemic attack/cerebrovascular accident, *n* (%)	12 (3.4)	8 (6.6)	0.1
Inflammation			
High-sensitivity C-reactive protein (mg/L)	1.8 (0.7–4.3)	2.8 (1.2–5.9)	0.007
Lipids			
Total cholesterol (mmol/L)	5.7 ± 1.1	5.6 ± 1.0	0.2
LDL (mmol/L)	3.7 ± 1.0	3.4 ± 0.9	0.007
HDL (mmol/L)	1.1 ± 0.3	1.0 ± 0.7	<0.001
Triglycerides (mmol/L)	1.8 (1.3–2.4)	2.4 (1.7–3.2)	<0.001
Use of statin at index, *n* (%)	165 (45.1)	71 (58.7)	0.009
Glucose homeostasis			
Glucose (mmol/L)	4.5 ± 0.6	4.8 ± 0.8	<0.001
Insulin (μmol/L)	9.4 (7.0–12.4)	15.0 (11.1–21.1)	<0.001
HOMA	1.81 (1.29–2.52)	3.28 (2.31–4.39)	<0.001
Proinsulin (pmol/L)	13.8 (9.6–17.9)	36.4 (28.4–51.5)	<0.001
Proinsulin/insulin ratio	1.4 (1.0–1.9)	2.6 (1.7–3.4)	<0.001
Family history of diabetes: parent or sibling with diabetes, *n* (%)	86 (23)	32 (26)	0.5
Renal allograft function			
Serum creatinine concentration (μmol/L)	134.0 (111.8–163.3)	146.0 (123.0–174.0)	0.007
Urinary creatinine excretion	11.9 (9.5–14.4)	11.9 (9.8–13.9)	1.0
Creatinine clearance (mL/min)	60.0 (47.0–78.0)	57.0 (43.0–71.5)	0.04
Urinary protein excretion (g/24 h)	0.2 (0.0–0.5)	0.3 (0.1–0.5)	0.1
Proteinuria, *n* (%)	98 (26.8)	36 (29.8)	0.5
Transplantation and history			
Number of transplantations >1, *n* (%)	39 (10.7)	12 (9.9)	0.2
Time after transplantation (years)	6.4 (2.9–12.2)	5.7 (2.2–12.2)	0.8
Dialysis duration (months)	26 (12–49)	33 (19–49)	0.06
Immunosuppression			
Calcineurine inhibitor, *n* (%)	286 (78.1)	93 (76.9)	0.8
Cyclosporine, *n* (%)	234 (61.6)	82 (67.8)	0.3

Continued on p. 1929

Table 1—*Continued*

	Quartiles of proinsulin		
	1–3 (*N* = 366)	4 (*N* = 121)	*P*
Cyclosporine (trough level, μg/L)	108 (80–138)	104 (77–149)	0.9
Tacrolimus, *n* (%)	57 (15.6)	11(9.1)	0.07
Tacrolimus (trough level, μg/L)	8.7 (6.4–10.2)	8.6 (6.0–9.7)	0.9
Proliferation inhibitor, *n* (%)	278 (87)	72 (72)	0.4
Azathioprine, *n* (%)	124 (33.9)	38 (31.4	0.6
Mycophenolate mofetil, *n* (%)	154 (42.1)	49 (40.5)	0.8
Sirolimus, *n* (%)	8 (2.2)	2 (1.7)	0.7
Sirolimus (trough level, μg/L)	9.0 ± 4.9	7.3 ± 6.3	0.7
Prednisolone dose (mg/day)	9.1 ± 1.4	9.3 ± 1.3	0.3

Data are represented as mean ± SD or median (95% CI). Differences were tested by ANOVA or Kruskal-Wallis test for continuous variables and with χ^2 for categorical variables.

(75%) fulfilled the criteria for MS. Prevalence of MS was 101 (86%) in the highest quartile of proinsulin compared with 208 (57%) in the lowest three quartiles ($P < 0.001$).

NODAT developed during median follow-up for 10.1 (interquartile range, 9.7–10.4) years in 76 (16%) RTR. Incidence of NODAT was 42 (35%) in the highest quartile compared with 34 (9%) in the lowest three quartiles of proinsulin ($P < 0.001$) (Fig. 1). Cumulative percentages of NODAT at 1, 3, 5, and 10 years after baseline were 1.2, 4.6, 7.4 and 14.8%, respectively.

Subsequently, we proceeded with prospective analyses for proinsulin and development of NODAT during follow-up. Proinsulin (hazard ratio [HR], 2.29; 95% CI, 1.85–2.83; $P < 0.001$) strongly predicted NODAT development in RTR in univariate analyses. Multivariate Cox regression analyses for proinsulin and NODAT development are shown in Table 2. Adjustment for age and sex did not materially influence the associations (model 2). We adjusted for use of cyclosporine, tacrolimus, and prednisolone dose in model 3. This adjustment also did not materially influence the association of proinsulin with NODAT development. Of note, use of tacrolimus was significantly associated with NODAT development (HR, 2.84; 95% CI, 1.37–5.89; $P = 0.005$) in this Cox regression model. This was independent of proinsulin, age, sex, use of cyclosporine, and prednisolone dose. We found no significant association of use of cyclosporine with development of NODAT. In further analyses in which we additionally adjusted for trough levels of tacrolimus and cyclosporine (model 4), we found no additional association of trough levels of tacrolimus with NODAT. However, trough levels of cyclosporine were associated with increased risk for higher concentrations (HR, 1.07; 95% CI, 1.01–1.13; $P = 0.02$). In further analyses, it appeared that the association between proinsulin and NODAT was independent of HOMA (model 5). Interestingly, in this model, HR for HOMA also was associated with NODAT independent of proinsulin (HR, 1.23; 95% CI, 1.09–1.40; $P = 0.001$). Adjustments for factors of MS (model 6) slightly weakened the association, but proinsulin remained independently associated with NODAT. Waist circumference (HR, 1.02; 95% CI, 1.01–1.04; $P = 0.01$), triglycerides (HR, 1.21; 95% CI, 1.05–1.40; $P = 0.01$), and glucose (HR, 2.26; 95% CI, 1.63–3.11; $P < 0.001$) were significantly associated with NODAT, independent of proinsulin.

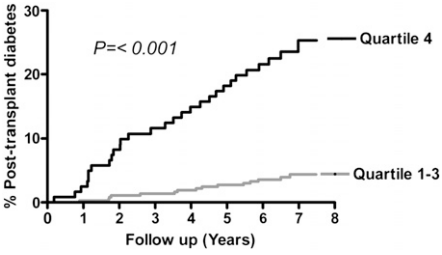

Figure 1—*Kaplan-Meier curve of de novo diabetes in quartiles of proinsulin tested with log-rank test (P < 0.001). Cut-off points for quartiles of proinsulin were as follows: quartiles 1–3, 2.4–24.4 (pmol/L), and quartile 4, >24.5 (pmol/L).*

Evaluation of prognostic value of proinsulin is summarized in Table 3. Harrell c-index of discrimination improved from 0.71 (interquartile range, 0.65–0.77) to 0.80 (interquartile range, 0.75–0.85 [see Table 3]; $P < 0.01$) after adding proinsulin to a clinical prediction model including sex, smoking, waist circumference, hypertension, and family history of diabetes (16). IDI analysis shows that the clinical risk score with proinsulin predicted NODAT more accurately than the clinical risk score alone. We found similar results when HOMA or glucose was added to the clinical model. When proinsulin was added on top of glucose, IDI was positive and remained significant. These results show that proinsulin is a promising biomarker for predicting NODAT beyond established clinical risk predictors in RTR.

CONCLUSIONS—Our study shows that proinsulin is an independent predictor of NODAT in RTR. Proinsulin levels predicted development of NODAT in RTR after adjustment for risk factors for NODAT. Our findings emphasize the importance of β-cell dysfunction in the pathophysiology of NODAT in RTR.

In our study, adjustment for MS (waist circumference, triglycerides, HDL cholesterol, blood pressure, and glucose concentration) attenuated the association of proinsulin with NODAT. This supports the notion that MS and particularly waist circumference, triglycerides, and glucose, partly contribute to this association. Factors of the MS could be modified by regular physical activity and a healthy diet, which shows the importance of lifestyle interventions after renal transplantation. The association between proinsulin and NODAT was independent of HOMA, which suggests that the relationship is driven by β-cell dysfunction.

It has become clear that both insulin resistance and β-cell dysfunction are present early in the natural history of diabetes. There is a hyperbolic

Table 2—*Proinsulin independently predicts NODAT in RTR*

Cox regression model	HR (95% CI)	*P*
1	2.29 (1.85–2.83)	<0.001
2	2.26 (1.83–2.81)	<0.001
3	2.43 (1.94–3.04)	<0.001
4	2.44 (1.95–3.04)	<0.001
5	1.76 (1.34–2.32)	<0.001
6	1.58 (1.25–1.99)	<0.001

Model 1, univariate analyses; model 2, model 1 + adjustment for recipient age and sex; model 3, model 2 + adjustment for cyclosporine, tacrolimus, and prednisolone dose; model 4, model 3 + adjustment for trough levels of cyclosporine and tacrolimus; model 5, model 2 + adjustment HOMA; model 6, model 2 + adjustment all components of the MS (waist circumference, triglycerides, HDL cholesterol, blood pressure, and glucose concentration).

relationship between insulin sensitivity and insulin secretion that depends on a negative feedback loop. Pancreatic β-cells compensate for changes in insulin sensitivity (18,19). In healthy subjects, plasma glucose levels are maintained near to normal, even with low insulin sensitivity, as a consequence of a compensatory increase in insulin secretion. RTR are more insulin-resistant than the general population. Oterdoom et al. (20) showed that obesity, waist-to-hip ratio, and prednisolone treatment are the predominant determinants of insulin resistance after transplantation. Furthermore, proinsulin can be used as a marker of pancreatic β-cell dysfunction. In this light, increased circulating levels of proinsulin are seen as a marker of β-cell stress when insulin demands required for maintenance of glycemic control are relatively high for the prevailing β-cell capacity. This is accompanied by increased "spill-over" of proinsulin (5).

Calcineurin inhibitors impair insulin secretion, produce β-cell toxicity, cause insulin resistance, and thereby contribute to an increased risk for NODAT (21–23). Various studies comparing cyclosporine and tacrolimus showed that use of cyclosporine is associated with a significantly lower incidence of NODAT than tacrolimus after renal transplantation (3,8,24). Interestingly, proinsulin levels tended to be lower in RTR receiving tacrolimus, despite the fact that use of tacrolimus was significantly associated with increased risk for NODAT development. Use of tacrolimus increased the risk for NODAT by almost three-fold (HR, 2.84; 95% CI, 1.37–5.89; $P = 0.005$). This observation could point to the known interference of tacrolimus with insulin production by pancreatic β-cells at the level of synthesis rather than at the level of conversion of proinsulin to insulin (21–23). The low variation in steroid doses in the population we investigated did not allow for us to find a relationship between steroid dose and NODAT.

Based on the results from the United Kingdom Prospective Diabetes Study, it was suggested that β-cell dysfunction was reduced up to 50% at time of diagnosis (25). Loss of β-cell dysfunction starts many years before diagnosis. Therefore, proinsulin can be used to identify patients at risk for NODAT development several years later. Elevated levels of proinsulin can be present despite normal glucose values. Proinsulin can still bind to the insulin receptor and has a glucose-lowering effect of 10–20% compared with insulin (26). Because of this minor but evident effect, patients with β-cell dysfunction do not always have diabetes diagnosed. Although no cut-off points for proinsulin are described in the literature, there is one study in which cut-off values are given for defining insulin resistance. Data from the IRIS-II (study on insulin resistance and insulin sensitivity) show that elevated proinsulin levels (>10 pmpl/L) are a good indirect marker for insulin resistance (27). In our study, 80% of the RTR had proinsulin levels above this threshold of >10 pmpl/L. This may reflect the fact that β-cell function is impaired in almost all RTR because of increased metabolic demands on the β-cells and the chronic exposure to immunosuppressive drugs, which places RTR at high risk for development of NODAT (2).

Sharif et al. (28) validated important insulin resistance indexes in RTR using tacrolimus. Rodrigo et al. (29) analyzed the performance of two general population risk scores for prediction of diabetes in RTR. None of these three evaluations included a marker of β-cell dysfunction. Chakkera et al. (30) recently developed a pretransplant risk score for the prediction of post-transplant diabetes. Also, in this study, no marker of β-cell dysfunction was included. The performance of the risk score developed by Chakkera et al. (30) was modest, with areas under receiver-operating curves varying between 0.70 and 0.72. Our study is the first to include a marker of β-cell dysfunction in addition to insulin resistance indexes in the prediction of NODAT in RTR. In our analyses, we found that both proinsulin as a marker of β-cell dysfunction and HOMA as a marker for insulin resistance are independently associated with increased risk for NODAT. The prediction model with proinsulin had good discrimination, showing that 80% of the RTR were adequately classified as at risk for NODAT. Integrated discrimination improved by 8% by adding proinsulin to the model. Proinsulin is a promising marker for early detection of patients at risk for NODAT and, possibly, future studies also may identify it as useful in the clinic to monitor β-cell function early after transplantation. Monitoring β-cell functions could allow for early intervention and treatment strategies to preserve β-cell function. Interestingly, Hecking et al. (31) recently showed in a randomized controlled study that basal insulin therapy may be a good strategy to reduce HbA_{1c} and may decrease the incidence of NODAT, presumably by protection of the β-cells. Patients were randomized to immediate postoperative isophane insulin (treatment group) or short-acting insulin

Table 3—*Additive value of proinsulin for the prediction risk of development of NODAT*

	C-statistic (95% CI)	*P* for change in C-statistic	IDI	*P*
Model 1	0.71 (0.65–0.77)	—	—	—
Model 1 + proinsulin	0.80 (0.75–0.85)	<0.01	0.077	<0.01
Model 1 + HOMA	0.77 (0.71–0.82)	<0.01	0.077	<0.01
Model 1 + glucose	0.78 (0.73–0.84)	<0.01	0.075	(<0.01)
Model 1 + glucose and proinsulin	0.80 (0.75–0.85)	0.06	0.046	(<0.01)
Model 1 + proinsulin and glucose	0.80 (0.75–0.85)	0.06	0.038	(<0.01)

Model 1 uses clinical model, including sex, smoking, waist circumference, hypertension, and family history of diabetes.

with or without oral antidiabetic agents (standard care). The treatment group had 73% lower odds for NODAT and HbA_{1c} was 0.38% lower than in the control group. Besides pharmacological strategies, lifestyle interventions could play an important role in prevention of NODAT beyond the first year after transplantation. Exercise training decreases insulin resistance and the risk of diabetes in the general population, and it can be assumed that it has similar effects in RTR. Sharif et al. (32) showed that lifestyle modification is beneficial for high-risk RTR with glucose intolerance. Intensive lifestyle modification (dietitian, exercise program, and weight loss advice) resulted in 15% improvement in 2-h postprandial glucose (32). Lifestyle interventions targeting physical activity and diet after transplantation as well as individualized choice of immunosuppressive agents could help in the prevention of NODAT.

The strength of our study is its prospective design. RTR in this study were closely monitored through regular checkups at our clinic, which provide complete information on patient status. Our study population is a cross-cut of all the RTR who visited our outpatient clinic, giving a variation of RTR with different times after transplantation and including stable RTR late after transplantation. However, our study is limited by the heterogeneousness of our study population, with variable time after transplantation and immunosuppressant medication. The healthy survivor effect is another drawback of our study. We used multivariate Cox regression modeling to adjust for confounders of NODAT. This modeling cannot fully correct for the fact that RTR without NODAT late after transplantation may be a healthier group. Future studies could investigate whether proinsulin levels measured earlier after transplantation also predict NODAT.

Proinsulin is strongly related to development of NODAT in RTR. Our results highlight the role of β-cell dysfunction in the pathophysiology of NODAT in RTR. Considering that the development of NODAT is associated with a higher risk of complications and worse survival, identifying RTR at increased risk for development of NODAT is needed. Proinsulin as a marker of β-cell dysfunction has potential value for identification of RTR at increased risk for NODAT. Prevention of NODAT by lifestyle interventions, early identification of patients at risk, and choice of immunosuppressive medication are all important to control and manage NODAT in RTR.

Acknowledgments—This research was performed within the framework of the Centre for Translational Molecular Medicine (CTMM; www.ctmm.nl), project PREDICCt (Grant 01C-104), and was supported by the Netherlands Heart Foundation, Dutch Diabetes Research Foundation, and Dutch Kidney Foundation.

No potential conflicts of interest relevant to this article were reported.

D.M.Z. contributed to data analyses and writing of the manuscript. E.C., J.D., R.P.S., R.O.B.G., G.N., and S.J.L.B. were responsible for intellectual contributions. D.M.Z. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Kuypers DR, Claes K, Bammens B, Evenepoel P, Vanrenterghem Y. 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
2. Chadban S. New-onset diabetes after transplantation—should it be a factor in choosing an immunosuppressant regimen for kidney transplant recipients. Nephrol Dial Transplant 2008;23:1816–1818
3. Kasiske BL, Snyder JJ, Gilbertson D, Matas AJ. Diabetes mellitus after kidney transplantation in the United States. Am J Transplant 2003;3:178–185
4. Bee YM, Tan HC, Tay TL, Kee TY, Goh SY, Kek PC. Incidence and risk factors for development of new-onset diabetes after kidney transplantation. Ann Acad Med Singapore 2011;40:160–167
5. Pfützner A, Pfützner AH, Larbig M, Forst T. Role of intact proinsulin in diagnosis and treatment of type 2 diabetes mellitus. Diabetes Technol Ther 2004;6:405–412
6. Bloom RD, Crutchlow MF. New-onset diabetes mellitus in the kidney recipient: diagnosis and management strategies. Clin J Am Soc Nephrol 2008;3(Suppl 2):S38–S48
7. Luan FL, Steffick DE, Ojo AO. New-onset diabetes mellitus in kidney transplant recipients discharged on steroid-free immunosuppression. Transplantation 2011; 91:334–341
8. Cole EH, Johnston O, Rose CL, Gill JS. 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
9. Pfützner A, Pfützner AH, Kann PH, et al. Clinical and laboratory evaluation of a new specific ELISA for intact proinsulin. Clin Lab 2005;51:243–249
10. Zelle DM, Corpeleijn E, van Ree RM, et al. Markers of the hepatic component of the metabolic syndrome as predictors of mortality in renal transplant recipients. Am J Transplant 2010;10:106–114
11. Oterdoom LH, van Ree RM, de Vries AP, et al. Urinary creatinine excretion reflecting muscle mass is a predictor of mortality and graft loss in renal transplant recipients. Transplantation 2008;86:391–398
12. Oterdoom LH, de Vries AP, van Son WJ, et al. Validation of insulin resistance indexes in a stable renal transplant population. Diabetes Care 2005;28:2424–2429
13. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation 2002;106:3143–3421
14. Davidson J, Wilkinson A, Dantal J, et al; International Expert Panel. New-onset diabetes after transplantation: 2003 International consensus guidelines. Proceedings of an international expert panel meeting. Barcelona, Spain, 19 February 2003. Transplantation 2003;75(Suppl): SS3–SS24
15. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care 2003;26(Suppl 1):S5–S20
16. Balkau B, Lange C, Fezeu L, et al. Predicting diabetes: clinical, biological, and genetic approaches: data from the Epidemiological Study on the Insulin Resistance Syndrome (DESIR). Diabetes Care 2008; 31:2056–2061
17. Harrell FE. *Regression modeling strategies: with applications to linear models, logisticregression, and survival analysis*. 2001. New York, Springer.
18. Kahn SE. The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of Type 2 diabetes. Diabetologia 2003;46:3–19
19. Bergman RN, Ader M, Huecking K, Van Citters G. Accurate assessment of beta-cell function: the hyperbolic correction. Diabetes 2002;51(Suppl 1):S212–S220
20. Oterdoom LH, de Vries AP, Gansevoort RT, et al. Determinants of insulin resistance in renal transplant recipients. Transplantation 2007;83:29–35
21. Ajabnoor MA, El-Naggar MM, Elayat AA, Abdulrafee A. Functional and morphological study of cultured pancreatic islets treated with cyclosporine. Life Sci 2007; 80:345–355
22. Redmon JB, Olson LK, Armstrong MB, Greene MJ, Robertson RP. Effects of tacrolimus (FK506) on human insulin gene

expression, insulin mRNA levels, and insulin secretion in HIT-T15 cells. J Clin Invest 1996;98:2786–2793
23. Øzbay LA, Smidt K, Mortensen DM, Carstens J, Jørgensen KA, Rungby J. Cyclosporin and tacrolimus impair insulin secretion and transcriptional regulation in INS-1E beta-cells. Br J Pharmacol 2011;162:136–146
24. Webster AC, Woodroffe RC, Taylor RS, Chapman JR, Craig JC. Tacrolimus versus ciclosporin as primary immunosuppression for kidney transplant recipients: meta-analysis and meta-regression of randomised trial data. BMJ 2005;331:810
25. U.K. Prospective Diabetes Study Group. U.K. prospective diabetes study 16. Overview of 6 years' therapy of type II diabetes: a progressive disease. Diabetes 1995;44:1249–1258
26. Galloway JA, Hooper SA, Spradlin CT, et al. Biosynthetic human proinsulin. Review of chemistry, in vitro and in vivo receptor binding, animal and human pharmacology studies, and clinical trial experience. Diabetes Care 1992;15:666–692
27. Pfützner A, Kunt T, Hohberg C, et al. Fasting intact proinsulin is a highly specific predictor of insulin resistance in type 2 diabetes. Diabetes Care 2004;27:682–687
28. Sharif A, Ravindran V, Moore RH, et al. Insulin resistance indexes in renal transplant recipients maintained on tacrolimus immunosuppression. Transplantation 2010;89:327–333
29. Rodrigo E, Santos L, Piñera C, et al. Prediction at first year of incident new-onset diabetes after kidney transplantation by risk prediction models. Diabetes Care 2012;35:471–473
30. Chakkera HA, Weil EJ, Swanson CM, et al. Pretransplant risk score for new-onset diabetes after kidney transplantation. Diabetes Care 2011;34:2141–2145
31. 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
32. Sharif A, Moore R, Baboolal K. Influence of lifestyle modification in renal transplant recipients with postprandial hyperglycemia. Transplantation 2008;85:353–358