HDL Cholesterol Efflux Predicts Incident New-Onset Diabetes After Transplantation (NODAT) in Renal Transplant Recipients Independent of HDL Cholesterol Levels

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In renal transplant recipients (RTRs), new-onset diabetes after transplantation (NODAT) is a frequent and serious complication limiting survival of graft and patient. However, the underlying pathophysiology remains incompletely understood. In vitro and in preclinical models, HDL can preserve β-cell function, largely by mediating cholesterol efflux, but this concept has not been evaluated in humans. This study investigated whether baseline cholesterol efflux capacity (CEC) in RTRs is associated with incident NODAT during follow-up. This prospective longitudinal study included 405 diabetes-free RTRs with a functioning graft for >1 year. During a median (interquartile range) follow-up of 9.6 (6.6–10.2) years, 57 patients (14.1%) developed NODAT. HDL CEC was quantified using incubation of human macrophage foam cells with apolipoprotein B–depleted plasma. Baseline CEC was significantly lower in patients developing NODAT during follow-up (median 6.84% [interquartile range 5.84–7.50%]) compared with the NODAT-free group (7.44% [6.46–8.60%]; *P* = 0.001). Kaplan-Meier analysis showed a lower risk for incident NODAT with increasing sex-stratified tertiles of HDL efflux capacity (*P* = 0.004). Linear regression analysis indicated that CEC is independently associated with incident NODAT (*P* = 0.04). In Cox regression analyses, CEC was significantly associated with NODAT (hazard ratio 0.53 [95% CI 0.38–0.76]; *P* < 0.001), independent of HDL cholesterol levels (*P* = 0.015), adiposity (*P* = 0.018), immunosuppressive medication (*P* = 0.001), and kidney function (*P* = 0.01). Addition of CEC significantly improved the predictive power of the Framingham Diabetes Risk Score (*P* = 0.004). This study establishes HDL CEC as a strong predictor of NODAT in RTRs, independent of several other recognized risk factors.

Diabetes constitutes a prime risk factor for end-stage renal disease (1,2). However, patients with end-stage renal disease free of diabetes also suffer a substantial risk to develop diabetes after receiving a kidney graft (3,4). Although the incidence of new-onset diabetes after transplantation (NODAT) is estimated to be high, some studies report values up to 50%, the pathogenesis of NODAT is still incompletely understood (4,5). Partly, similar risk factors associated with type 2 diabetes in the general population seem to play a role, and partly transplantation-specific impacting factors are held responsible (3–5). Of particular interest are conditions related to the metabolic syndrome such as obesity on the one hand and medication preventing graft rejection such as tacrolimus or corticosteroids on the other (4,5). NODAT itself is significantly associated with decreased patient survival and increased kidney graft failure (5,6). Still, prospective biomarkers helping to stratify patients at high or low risk for NODAT are scarce, and no algorithm to predict NODAT in posttransplant renal transplant recipients (RTRs) has been validated.

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The pathophysiology of type 2 diabetes and NODAT has two components, defects in peripheral insulin sensitivity and insufficient insulin secretion by the pancreatic β-cells to meet demand (4,5). The insulin-producing pancreatic β-cell is particularly sensitive to disturbances in cholesterol homeostasis. LDL cholesterol (LDL-C) uptake into β-cells has been associated with cellular dysfunction, apoptosis, and failure to secrete sufficient amounts of insulin (7,8). Clinically, this might be reflected by a higher type 2 diabetes risk in subjects receiving statins that increase cellular LDL receptor expression (7–9). HDL particles, on the contrary, exert protective effects on β-cells in vitro (10). A key functionality of HDL in this respect is to induce cellular cholesterol efflux and thereby unload cholesterol from β-cells (7,11). This has been illustrated, for example, by the observation that subjects lacking ABCA1, a major cellular cholesterol efflux transporter, are at an increased risk of developing type 2 diabetes (12). In the general population, however, an association between low HDL cholesterol (HDL-C) mass levels and diabetes risk cannot be so firmly established, as indicated by a recent Mendelian randomization study (13). This discrepancy might also point toward an added clinical value of determining metrics of HDL function, such as cholesterol efflux. Next to cellular requirements, structure and composition of the cholesterol-receiving HDL particle are key factors determining the rate of efflux (7,11). Thus, specifically via their cholesterol efflux function, HDL particles might be able to modify the risk of developing type 2 diabetes or NODAT. However, no studies are currently available that prospectively evaluate this concept in RTRs. Therefore, the present work aims to establish whether baseline cholesterol efflux in RTRs is associated with incident NODAT during follow-up.

RESEARCH DESIGN AND METHODS

Study Design and Study Population

For inclusion into this prospective cohort study, all RTRs at the University Medical Center Groningen with a functioning allograft for >1 year after transplantation were eligible. Patient recruitment was carried out between August 2001 and July 2003. RTRs are subjected to a continuous follow-up system in the outpatient clinic with a declining frequency, as outlined in the American Society of Transplantation guidelines, ranging from twice a week immediately after hospital discharge to twice a year in the long-term course after transplantation (14). Patients with known systemic illnesses such as congestive heart failure, cancer other than cured skin cancer, endocrine disorders other than diabetes, or overt generalized infections were excluded. Of 847 eligible patients, 606 RTRs gave written informed consent to participate. Participants did not differ from the group that did not give consent with respect to age, sex, BMI, plasma creatinine, creatinine clearance, and proteinuria. A more comprehensive description of the study setup has been published previously (15–18). In order to study the future development of diabetes of the 606 included RTRs, 105 participants with already existing type 1 and 2 diabetes or impaired fasting glucose or using glucose-lowering drugs were excluded. Furthermore, for 92 of the remaining RTRs, no baseline material was available to determine HDL cholesterol efflux capacity (CEC). In addition, four RTRs were not included, because they received a mixed liver-kidney transplant. Clinical information regarding donors and recipients as well as transplant characteristics were obtained from the Groningen Renal Transplant Database. The study protocol complied with the Declaration of Helsinki and was approved by the Medical Ethical Institutional Review Board of the University Medical Center Groningen (METc 01/039).

End Point of the Study

The main outcome measure of this study is HDL CEC. The primary end point is incident NODAT during follow-up.

Measurements and Definitions

NODAT was defined according to the Expert Panel recommendations based on the 2003 American Diabetes Association criteria (19). The diagnosis was based on the following criteria: classic symptoms (unexplained weight loss, polydipsia, and polyuria), fasting (no caloric intake for at least 8 h) plasma glucose concentration >126 mg/dL (7.0 mmol/L), nonfasting plasma glucose concentration of >200 mg/dL (11 mmol/L), or the use of glucose-lowering medication (such medication was only prescribed to patients with manifest NODAT). Patients were categorized as having impaired plasma glucose, if fasting plasma glucose was >100 mg/dL (5.6 mmol/L) and <126 mg/dL (7.0 mmol/L). At all routine follow-up visits (see above), fasting glucose was determined using capillary glucose testing (glucose-oxidase method, YSI 2300 Stat Plus; YSI Incorporated, Yellow Springs, OH). In case plasma glucose was elevated, a confirmatory laboratory test of venous plasma was done on the subsequent day or upon the next visit, after which the diagnosis of NODAT was made. To establish/exclude diabetes at baseline, the same criteria were applied as described above; thus, also a confirmatory glucose measurement was required.

Waist circumference was measured on the skin midway between the iliac crest and the 10th rib. Blood pressure was measured three times in the supine position after at least 6-min rest using an automated device (OMRON M4; OMRON Healthcare Europe B.V., Hoofddorp, the Netherlands), and then the average of the three measurements was taken. BMI was calculated as weight (kilograms) divided by height in meters squared. Insulin resistance was calculated using HOMA of insulin resistance (HOMA-IR) according to the formula: HOMA-IR = glucose (mmol/L) × insulin (μU/mL)/22.5. HbA_{1c} was assessed by high-performance liquid chromatography (VARIANT Hemoglobin Testing System; Bio-Rad Laboratories, Hercules, CA).

Estimated glomerular filtration rate (eGFR) was calculated according to the Chronic Kidney Disease Epidemiology Collaboration equations (20).

Blood samples were drawn in the morning after an 8–12-h overnight fast. Total cholesterol, HDL-C, triglycerides, and plasma insulin were analyzed by routine clinical chemistry methods as detailed before (15). LDL-C was calculated using the Friedewald equation, and apolipoprotein A-I (apoA-I) was determined by immunoturbidimetry (COBAS INTEGRA System; Roche Diagnostics, Mannheim, Germany). Plasma hs-CRP was measured by ELISA (15). Plasma and urine creatinine concentrations were determined using a modified version of the Jaffé method (MEGA AU 510; Merck). Total urinary protein concentration was measured with the Biuret reaction (MEGA AU 510; Merck); proteinuria was defined as urinary protein excretion $\geq$0.5 g/24 h.

Determination of HDL CEC

For cholesterol efflux measurements, blood samples were collected in EDTA-containing tubes at time of inclusion into the study, immediately placed on ice, centrifuged at 4°C, and stored at −80°C. Efflux was determined following a previously validated protocol (15,21,22). Briefly, HDL was isolated by precipitating apolipoprotein B (apoB)–containing lipoproteins with polyethylene glycol (PEG 6000; Sigma-Aldrich, St. Louis, MO) in 10 mmol/L HEPES (pH 8.0) as detailed previously (15,21–24). In order to assess CEC, THP-1 human monocytes (ATCC, Manassas, VA) were cultured in 48-well plates in RPMI 1640 Glutamax Medium (Gibco, Carlsbad, CA) containing 10% FBS and penicillin (100 U/mL)/streptomycin (100 μg/mL) and then differentiated into macrophages by the addition of 100 nmol/L phorbol myristate acetate (Sigma-Aldrich) for 24 h. Macrophages were subsequently loaded with 50 μg/mL acetylated LDL and 1 μCi/mL ^{3}H-cholesterol (PerkinElmer, Boston, MA) for 24 h followed by overnight equilibration with RPMI 1640 Glutamax Medium containing 2% BSA (Sigma-Aldrich). After washing with PBS, efflux was determined by the addition of 2% of individual apoB-depleted plasma samples for 5 h. Then medium was collected and centrifuged in a tabletop centrifuge (Andreas Hettich GmbH & Co, Tuttlingen, Germany) for 5 min at 10,000 rpm to pellet cellular debris, and radioactivity was determined in an aliquot by liquid scintillation counting (Packard 1600CA Tri-Carb; Packard Instrument Co, Meriden, CT). To the cells, 0.1 mol/L NaOH was added for at least 30 min, and then radioactivity remaining in the cells was determined. Efflux per well was calculated as the percentage of counts released into the medium related to the total dose of radioactivity initially present (counts recovered within the medium added to the counts recovered from the cells). Values obtained from control cells without added HDL were subtracted to correct for unspecific efflux. Cholesterol efflux measurements were carried out in duplicate and in all respective patient samples at the same time to limit potential variation due to different assay conditions. To be able to correct for potential plate-to-plate variation, the same apoB-depleted control plasma was included on each plate at four different concentrations. In this assay, almost all of the complete biological activity (>95%) of apoB-depleted plasma for cholesterol efflux is dependent on the presence of HDL, and freezing of plasma samples has no impact on the results (22). The intra-assay coefficient of variation of this method was previously determined to be 5.4% and the interassay coefficient of variation 7.9% (15,25).

Statistical Analysis

A P value of <0.05 was considered statistically significant. All statistical analyses were performed using the Statistical Package for the Social Sciences version 24 (IBM). All variables were checked for normality. Data with skewed distribution are expressed as median (interquartile range), and data with normal distribution are expressed as mean ± SD. Absolute numbers (percentages) are given for categorical variables. For variables with a skewed distribution, logarithmic transformation was used in order to reach normality criteria. The development of NODAT was visualized using Kaplan-Meier curves comparing all tertiles of efflux capacity; statistical significance was tested with the log rank (Mantel-Cox) test. In order to investigate parameters independently associated with NODAT, all characteristics with a $P \leq 0.1$ across sex-stratified cholesterol efflux tertiles were entered into a stepwise multivariate linear regression model with backward elimination. To determine if cholesterol efflux was prospectively associated with the risk of NODAT, Cox proportional hazards regression analysis was performed. In order to adjust for potential confounders, different models were made as indicated. Models were made based on associations ($P < 0.1$) of respective baseline characteristics with efflux (Table 1). Subsequently, the predictive capacity of cholesterol efflux was assessed using logistic regression analysis. Therefore, first, the Framingham Diabetes Risk Score (26) was calculated for all participants, and in a subsequent step, the performance of that diabetes prediction score was evaluated without and after the addition of cholesterol efflux.

RESULTS

Baseline Demographic Characteristics

In this prospective longitudinal study, HDL CEC was determined in 405 included RTRs (median [interquartile range] age 51.5 [42.2–59.4] years; 55.8% male), all free of diabetes at baseline. First, patients were divided into sex-stratified tertiles according to cholesterol efflux. The median efflux values in the respective tertiles were, first, 5.9% (5.4–6.4%); second, 7.2% (6.8–8.0%); and third, 9.0% (8.1–10.0%). Baseline characteristics among the tertiles are given in Table 1. Higher efflux values were associated with higher age and lower BMI, waist circumference, plasma insulin levels, and HOMA-IR. Further, patients with better graft function, determined as serum

Table 1—Baseline patient characteristics according to sex-stratified tertiles of cholesterol efflux capacity (n = 405)

Variable	Tertiles of sex-stratified HDL CEC (%)			
	T1 (low tertile)	T2 (middle tertile)	T3 (high tertile)	*P* value for trend
HDL CEC (%)	5.9 (5.4–6.4)	7.2 (6.8–8.0)	9.0 (8.1–10.0)	
General characteristics				
Age (years)	49.8 (41.5–58.0)	51.1 (40.4–58.9)	55.5 (45–61.5)	0.003
Male sex (%)	56.3	55.1	60.0	0.981
Smoking status (%)				
Never smoker	37	32.4	31.3	0.572
Former smoker	40.7	44.9	45.5	0.693
Current smoker	22.2	22.1	22.4	0.998
Alcohol consumption (%)				0.687
None	46.7	50	44	
>4 units/day	0.7	0.7	2.2	
1–3 units/day	11.1	14.7	13.4	
2–7 units/week	23.7	22.1	25.4	
1–4 units/month	17.8	11	13.4	
Body composition				
BMI (kg/m^2)	26.1 (23.5–29.2)	25.4 (23.1–27.6)	24.2 (22.6–26.6)	0.002
Waist circumference (cm)	98.6 ± 13.7	95.6 ± 12.6	92.4 ± 12.4	<0.001
Transplant history				
Time since renal transplantation (years)	5.8 (2.1–9.2)	5.3 (2.4–10.6)	8.3 (4.1–13.8)	0.001
Deceased donor (%)	84.4	84.6	89.6	0.383
Donor age (years)	41 (24–52)	41 (23–52)	32 (23–50)	0.363
Dialysis duration (months)	26 (13–51)	27.5 (12.3–45)	30 (13.8–52.3)	0.363
Renal allograft function				
Serum creatinine (μmol/L)	150 (121–183)	133 (112–157)	126 (106–148)	<0.001
eGFR (mL/min/1.73 m^2)	42.3 ± 15.3	49.4 ± 15.8	49.9 ± 15.5	<0.001
Urinary albumin-to-creatinine ratio	15.4 (5.2–50.7)	8.0 (3.4–43.2)	13.7 (3.8–68.6)	0.056
Inflammatory markers				
hs-CRP	2.1 (0.9–4.6)	1.8 (0.7–4.3)	1.7 (0.7–3.5)	0.097
Blood pressure				
Diastolic blood pressure (mmHg)	90 ± 10	89 ± 9	90 ± 10	0.454
Systolic blood pressure (mmHg)	149 (135–166)	147 (132–160)	152 (134–167)	0.204
Glucose homeostasis				
Fasted plasma glucose (mmol/L)	4.5 (4.1–4.9)	4.5 (4.1–4.9)	4.4 (4.0–4.8)	0.305
Plasma insulin (μmol/L)	11.5 (8.6–14.8)	10.9 (7.9–15.8)	9.0 (6.3–11.7)	<0.001
HbA_{1c} (%)	6.4 (5.8–6.7)	6.2 (5.7–6.6)	6.1 (5.6–6.6)	0.148
HbA_{1c} (mmol/mol)	46 (40–50)	44 (39–49)	43 (38–49)	0.148
Family history of diabetes: parent or sibling with diabetes (%)	13.3	8.8	9.7	0.466
Lipids and lipoproteins				
Total cholesterol (mmol/L)	5.4 ± 0.9	5.71 ± 1.25	5.82 ± 0.9	0.004
LDL-C (mmol/L)	3.6 (2.9–4.1)	3.5 (3.0–4.2)	3.5 (3.1–4.1)	0.824
HDL-C (mmol/L)	0.86 (0.71–0.99)	1.1 (0.96–1.2)	1.4 (1.2–1.5)	<0.001
ApoA-I	1.3 (1.2–1.5)	1.6 (1.4–1.7)	1.8 (1.6–2.0)	<0.001
ApoB	1.1 ± 0.2	1.1 ± 0.2	1.0 ± 0.2	0.006
Triglycerides (mmol/L)	2.1 (1.6–2.8)	2.0 (1.4–2.7)	1.6 (1.2–2.1)	<0.001
Medication use				
Antihypertensives (%)	92.6	80.9	82.1	<0.001
Statins (%)	48.1	48.5	51.5	0.377
Proliferation inhibitor (%)	80.7	75.7	70.1	0.129
Calcineurin inhibitor (%)	77.8	83.8	73.9	0.134
Tacrolimus (%)	15.6	14.0	8.2	0.161
Cyclosporine (%)	62.2	69.1	64.9	0.485
Prednisolone (mg/24 h)	10 (8.8–10)	10 (8.8–10)	10 (7.5–10)	0.015

Continuous data with a skewed distribution are given as median (interquartile range), and differences were assessed using the Kruskal-Wallis test. Normally distributed continuous data are presented as mean ± SD, and differences were tested using one-way ANOVA. Categorical data are given as %, and differences were analyzed by χ^2 test.

creatinine and eGFR, had a higher efflux capacity. In addition, HDL efflux function was positively associated with plasma total cholesterol, mainly explained by the relationship of efflux with HDL-C, while LDL-C was not different among the groups. Finally, patients with higher efflux values used less antihypertensives and had a higher daily dose of prednisolone, with no significant differences being detected with respect to the use of other immunosuppressive medications or statins.

Association of Cholesterol Efflux With Incident NODAT

During a median follow-up of 9.6 (6.6–10.2) years, a total of 57 patients (14.1%) developed NODAT. Baseline cholesterol efflux values were significantly lower in patients diagnosed with NODAT during follow-up compared with the group that remained NODAT free (6.84% [5.84–7.50%] vs. 7.44% [6.46–8.60%], respectively; $P = 0.001$), already indicating a possible association between better HDL efflux capacity at baseline and less incident NODAT during follow-up. Stepwise linear regression analysis entering all patient characteristics with a P value ≤ 0.1 across the sex-stratified cholesterol efflux tertiles was carried out to identify independent determinants of NODAT (Table 2). The results indicate that with decreasing order of strength plasma glucose concentration (odds ratio [OR] 2.65 [95% CI 1.68–4.17]; $P < 0.001$), plasma triglyceride concentration (OR 1.50 [1.15–2.00]; $P = 0.003$), notably cholesterol efflux (OR 0.80 [0.64–0.99]; $P = 0.04$), and BMI (OR 1.10 [1.02–1.19]; $P = 0.01$) were independently associated with the development of NODAT in RTRs, while, for example, HDL-C mass levels were not.

Next, NODAT incidence between tertiles of cholesterol efflux was compared using Kaplan-Meier analysis. Kaplan-Meier curves demonstrated a highly significant association of sex-stratified efflux percentage with the future development of NODAT (log-rank test: $P = 0.002$) (Fig. 1); during follow-up, 22.2% ($n = 30$) of the patients in the lowest tertile, 13.2% ($n = 18$) in the middle tertile, and 6.7% ($n = 9$) in the high efflux tertile developed NODAT.

Furthermore, COX proportional hazard analyses were carried out with the aim to determine the independent contribution of HDL CEC to the risk of developing NODAT (Table 3 and, for extended information, Supplementary Table 1). In univariate analysis (model 1), cholesterol efflux was significantly related to incident NODAT (hazard ratio [HR] 0.54 [95% CI 0.38–0.76]; $P < 0.01$). Further

Table 2—Variables that are determinants of NODAT

	OR	95% CI	*P* value
Glucose concentration (mmol/L)	2.65	1.68–4.17	<0.001
Triglyceride concentration (mmol/L)	1.50	1.15–2.00	0.003
Cholesterol efflux (%)	0.80	0.64–0.99	0.04
BMI (kg/m^2)	1.10	1.02–1.19	0.01

Variables are listed in decreasing order of strength of association according to the OR.

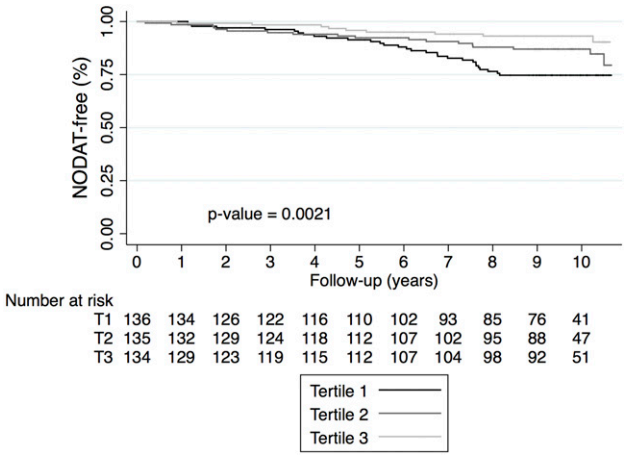

Figure 1—Kaplan-Meier analysis for cholesterol efflux and NODAT. The corresponding *P* value was obtained from log-rank tests.

adjustment for age and sex (model 2) strengthened this association (HR 0.53 [0.38–0.76]; $P < 0.001$). With the further addition of BMI and waist circumference to the multivariate analysis (model 3), efflux was still significantly associated with NODAT (HR 0.65 [0.45–0.93]; $P = 0.018$). Also, with adding time since renal transplantation (model 4, HR 0.54 [0.38–0.77]; $P = 0.01$), eGFR (model 5, HR 0.53 [0.37–0.76]; $P = 0.01$), or plasma insulin and HOMA-IR (model 6, HR 0.56 [0.39–0.81]; $P = 0.02$) to model 2, the association between efflux and NODAT remained significant. Interestingly, model 7 demonstrates that taking account of a number of relevant lipid parameters, including total cholesterol, HDL-C, apoA-I, apoB, and triglycerides, also did not change the significant association of cholesterol efflux and incident NODAT (HR 0.52 [0.31–0.88]; $P = 0.015$). Further adjustment of model 2 for a number of relevant immunosuppressive medications used by RTRs did not change the conclusions reached from the other models (model 8, HR 0.53 [0.37–0.76]; $P = 0.001$). Finally, adding statin use did not weaken the association (model 9, HR 0.54 [0.38–0.76]; $P < 0.001$). For further adjustments for additional covariates including individual immunosuppressive medications as well as the independent determinants of NODAT identified by the linear regression analysis, see Supplementary Table 1.

Next, logistic regression analyses were carried out to explore whether addition of cholesterol efflux would add to a classical diabetes prediction model. For this purpose, we chose the Framingham Diabetes Risk Score, which, although not fully validated, has been indicated to be of value for the prediction of NODAT in RTRs (26). As shown in Table 4, also in our cohort, the Framingham Diabetes Risk Score was useful in predicting NODAT (OR 1.13 [1.08–1.18]; $P < 0.001$). Interestingly, including cholesterol efflux significantly improved the predictive capacity of the Framingham Diabetes Risk Score, further

Table 3—Cox regression analysis to determine HRs for NODAT incidence by CEC

	HR (95% CI)	*P* value
Model 1	0.54 (0.38–0.76)	0.01
Model 2	0.53 (0.38–0.76)	<0.001
Model 3	0.65 (0.45–0.93)	0.025
Model 4	0.54 (0.38–0.77)	0.01
Model 5	0.53 (0.37–0.75)	<0.001
Model 6	0.56 (0.39–0.81)	0.02
Model 7	0.52 (0.31–0.88)	0.015
Model 8	0.53 (0.37–0.76)	0.004
Model 9	0.54 (0.38–0.76)	<0.001

Model 1: crude analysis; model 2: adjustment for age and sex; model 3: model 2 plus adjustment for BMI, waist circumference, and hs-CRP; model 4: model 2 plus adjustment for time since renal transplantation; model 5: model 2 plus adjustment for eGFR and urinary albumin-to-creatinine ratio; model 6: model 2 plus adjustment for serum concentration of insulin and HOMA-IR; model 7: model 2 plus adjustment for total cholesterol, HDL-C, apoA-I, apoB, and triglycerides; model 8: model 2 plus adjustment for use of proliferation inhibitors, calcineurin inhibitors, tacrolimus, cyclosporine, antihypertensives, and daily prednisolone dose; and model 9: model 2 plus adjustment for use of statins.

strengthening the potential clinical utility of efflux determinations (OR 0.74 [0.60–0.92]; change from previous step, $P = 0.004$).

Combined, these data demonstrate that in RTRs, cholesterol efflux at baseline is significantly associated with the future risk to develop NODAT, independent of a number of established and perceived risk factors. Importantly, also taking account of lipid and lipoprotein measurements determined in routine clinical chemistry evaluations such as mass HDL-C levels did not change this conclusion.

DISCUSSION

The results of this prospective study demonstrate that the cholesterol efflux function of HDL predicts NODAT in RTRs, independent of mass levels of the commonly determined biomarker HDL-C as well as a number of other relevant impacting factors. Of note, cholesterol efflux represents a recognized key metric of HDL functionality with a pathophysiological link to integrity and function of pancreatic β-cells (7–9). Thereby, these data emphasize the concept that clinically relevant information can be retrieved from HDL function studies.

With an incidence of up to 50% among RTRs diabetes-free at time of transplantation, NODAT is a frequent complication after renal transplantation (3–5). NODAT is associated with reduced graft survival as well as with an increased risk for infection and cardiovascular mortality, all contributing to decreased patient survival (4,5,27). In the course of the rapidly increasing overall incidence of type 2 diabetes, it is believed that NODAT will become an even more prominent clinical problem in the future (3–5). In addition to the impact on health and quality of life of the individual patient, NODAT also imposes significant costs on health care systems; in the U.S., it was estimated that within the first year after renal transplantation, NODAT causes costs of $12,000/patient and >$19,000 in the following year (28). Although NODAT thus represents a substantial burden, surprisingly little is known about its specific pathophysiology. It is recognized, however, that general factors relevant for type 2 diabetes play a role such as age, family history of diabetes, or previous glucose intolerance as well as specific factors associated with the underlying kidney disease before transplantation and, for example, the immunosuppressive medications mandatory following transplantation (3–5,27). With respect to the glycemic effects of immunosuppressive drugs, glucose tolerance testing indicated that, next to the known negative effect of glucocorticoids (4,5), tacrolimus reduces insulin secretion by pancreatic β-cells in a dose-dependent fashion contributing to hyperglycemia in kidney transplant recipients (29). Converting patients receiving renal transplantation from tacrolimus to cyclosporin A, in contrast, was associated with improved glucose metabolism parameters (30). An additional improvement in glycemic control was noted when RTRs were switched to a cyclosporin A–sparing immunosuppression by the use of mammalian target of rapamycin inhibitors such as everolimus (31). Mirroring the impact of general type 2 diabetes risk on NODAT pathogenesis, risk scores for the prediction of incident type 2 diabetes have been applied with some success in an attempt to provide a better prediction for NODAT. Models using both pretransplant data (including planned use of maintenance corticosteroids) (32) as well as early posttransplant clinical information (San Antonio Diabetes Prediction Model, Framingham Offspring

Table 4—Logistic regression analysis of the Framingham Diabetes Risk Score without and with the addition of HDL CEC measurements

			Change from previous step	
	OR (95% CI)	*P* value	Model χ^2	*P* value
Model 1		<0.001	30.5	NA
Framingham Diabetes Risk Score	1.13 (1.08–1.18)			
Model 2		<0.001	8.5	0.004
Framingham Diabetes Risk Score	1.12 (1.07–1.18)			
Cholesterol efflux (%)	0.74 (0.60–0.92)			

Study-Diabetes Mellitus) were able to predict NODAT to a certain extent (26). Currently, no single predictive biomarker is available to assign individual risk to patients to help identify RTRs who need early therapeutic intervention. In our study, the linear association of blood glucose and triglycerides levels with NODAT was stronger than the association of HDL CEC with NODAT. Nonetheless, addition of CEC to the Framingham Diabetes Risk Score improved the prediction capacity for NODAT. Therefore, even though the overall effect size was relatively small, these data suggest that HDL CEC provides a useful additive biomarker for NODAT that is also mechanistically linked to diabetes pathophysiology.

In the cardiovascular field, studying HDL function, in particular cholesterol efflux, represents an emerging topic (11,33). Available data indicate that low cholesterol efflux is prospectively associated with increased incident cardiovascular events (23,24) in the general population and with chronic atherosclerosis–driven graft failure in RTRs (15). However, cholesterol is not only relevant for cardiovascular disease but also for both components that determine deranged glucose metabolism in type 2 diabetes/NODAT, namely increased peripheral insulin resistance and decreased functionality of pancreatic β-cells that fail to secrete sufficient amounts of insulin to meet the increased demand (4,5). Infusion of recombinant HDL particles has, for example, been shown to improve insulin sensitivity of skeletal muscle in humans (34). In contrast, pancreatic β-cells maintain a tight balance of their cholesterol content pertinent to their function (7,8). In vitro studies established that loading β-cells with cholesterol induces β-cell dysfunction and apoptosis, resulting in reduced insulin secretion, factors conceivably contributing to the pathogenesis of type 2 diabetes (7,8). On a population level, these data are mirrored by the now well-recognized increased risk of type 2 diabetes associated with statin therapy (9). Statins increase the cell surface expression of LDL receptor and thereby stimulate LDL uptake and consequently cholesterol loading of β-cells (8,9). In contrast, higher HDL-C levels are associated with decreased incident type 2 diabetes in the general population (35). In vitro, HDL particles were shown to protect β-cells from ER stress and apoptosis and to preserve their functionality (36,37). These beneficial biological effects appear intricately linked to the cholesterol efflux function of HDL particles (8,10). In vivo support for these findings comes from studies in mice and humans demonstrating that reduced expression or lack of the major cellular cholesterol export transporter ABCA1 is associated with reduced β-cell function and an increased type 2 diabetes risk (12,38). ABCA1 is established to interact with apoA-I, the class-defining apolipoprotein of HDL, to induce cellular cholesterol unloading (11,39). In addition, HDL also seems to have the capacity to improve peripheral insulin sensitivity as evidenced by studies using intravenous infusion of recombinant HDL particles (34). Taken together, although these combined data indicate that HDL can conceivably protect against type 2 diabetes, literature exploring this concept in humans is scarce, and especially no such results are currently available in the setting of NODAT, again emphasizing the novelty of our approach.

Several considerations with respect to potential limitations of our study should be taken into account. In general, statistical associations do not allow drawing firm conclusions on cause–effect relationships. Moreover, the interpretation of HDL function assays depends on the respective chosen assay conditions and on the HDL isolation method (11). Currently, no consensus has been reached with respect to standardization of these parameters, so that such assays are not fully comparable to, for example, values obtained by clinical chemistry determinations. In our work, we used an established assay that is using human macrophage foam cells, in which all efflux pathways are active (ABCA1, 47% contribution, determined by addition of probucol; SR-BI, 19% contribution, determined by addition of BLT-1; and ABCG1, 30% contribution, determined by the addition of probucol and BLT-1 [U.J.F.T., unpublished observations]); this offers certain advantages over, for example, murine J774 macrophages equilibrated with cholesterol label, in which efflux mainly depends on the ABCA1 system (40). HDL isolation was carried out with a protocol widely used in efflux studies (15,21,23,24). In addition, to minimize experimental variation, all efflux experiments were done at the same time with identical batches of cells and reagents. Next to technical, assay-related considerations, it should be pointed out that the data reported in this study are from a single center and that one of the inclusion criteria was to only study patients with a functioning allograft for >1 year. This was done because we wanted to exclude the impact of acute rejections, which are most prevalent during the first year, including ample adaptations of the immunosuppressive regimen and be able to evaluate the chronic long-term course after transplantation. However, that means, in turn, that our data do not allow drawing conclusions with respect to other factors that might play a role during the first posttransplant year. Although TransplantLines is one of the largest prospective renal transplant cohorts, a multicenter approach appears desirable to confirm our results. Such a follow-up study would also aid in identifying a simple and easy-to-measure biomarker that is reliably reflecting HDL function and thus has the potential to replace HDL-C in routine clinical determinations. The final goal of such experimental efforts would be to define therapeutic interventions targeted to improve HDL function and then test if NODAT can either be prevented or at least substantially delayed. Metabolomics hold great potential in this respect, not only for the identification of HDL-associated biomarkers, but also in general terms for the elucidation of molecules with the capacity to serve as predictors for the development of

cardiometabolic disease in patients with compromised kidney function (41).

In conclusion, the current study establishes HDL CEC as a predictor of NODAT in RTRs independent of a number of other recognized risk factors. HDL function measurements thus might be promising not only for improved diagnostics but also to better characterize a possible emerging target for therapeutic intervention.

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