Fruit and Vegetable Intake and Risk of Posttransplantation Diabetes in Renal Transplant Recipients

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OBJECTIVE

Posttransplantation diabetes mellitus (PTDM) contributes to risk for cardiovascular morbidity and mortality in renal transplant recipients (RTRs). In the general population, consumption of a diet containing few fruits and vegetables predisposes to type 2 diabetes. The role of diet as a potential modifiable risk factor for PTDM has not been explored. Our focus was to investigate the prospective associations of fruit and vegetable intake with risk of PTDM in stable RTRs.

RESEARCH DESIGN AND METHODS

We included 472 adult RTRs who had a functioning graft ≥1 year. Fruit and vegetable intake was assessed by using a 177-item food frequency questionnaire. PTDM was defined according the American Diabetes Association's diagnostic criteria for diabetes.

RESULTS

During 5.2 years of follow-up, 52 RTRs (11%) developed PTDM. Fruit intake was not associated with PTDM (hazard ratio [HR] 0.90 [95% CI 0.79–1.03] per ^{2}log g/day; P = 0.13), whereas vegetable intake was inversely associated with PTDM (HR 0.77 [95% CI 0.63–0.94] per ^{2}log g/day; P = 0.009). Mediation analyses revealed that ±50% of the association between vegetable intake and PTDM was mediated by variations in key components of the metabolic syndrome (i.e., HDL cholesterol, triglycerides, and waist circumference) as determined by the National Cholesterol Education Program's Adult Treatment Panel III Expert Panel.

CONCLUSIONS

In this study vegetable intake, but not fruit intake, was associated with lower risk of PTDM in RTRs, likely largely through beneficial effects on key components of the metabolic syndrome. These findings further support accumulating evidence that supports a recommendation of higher vegetable intake by RTRs.

Renal transplantation is the preferred treatment for patients with end-stage renal disease because it contributes to better quality of life and longer survival at lower costs than dialysis treatment (1,2). One of the main metabolic disorders following renal transplantation is posttransplantation diabetes mellitus (PTDM). The frequency of PTDM varies over time depending on changes in health care providers' vigilance for PTDM, changing definitions of PTDM, and changes in background immunosuppression; recent incidence estimates range from 7 to 39% at 1 year and from 10 to 30% at

3 years after transplantation (3). Renal transplant recipients (RTRs) who develop PTDM are at higher risk of complications, including cardiovascular morbidity and mortality compared with RTRs who do not develop PTDM (4–7). Pathophysiologically, PTDM is similar to type 2 diabetes in that both are characterized by increased insulin resistance and decreased insulin production (8,9). Although transplantation-specific risk factors for PTDM have been identified, including treatment with corticosteroids and use of tacrolimus as a calcineurin inhibitor, additional risk factors equivalent to those for type 2 diabetes in the general population are assumed to contribute to the risk for PTDM (9–11). Diet is regarded as one of the main modifiable risk factors for the development of type 2 diabetes; in particular, diets characterized by high fruit and vegetable intake have been associated with a lower risk for type 2 diabetes in several large general population–based studies (12–14). However, it remains unexplored whether fruit and vegetable intake might be associated with low risk of PTDM after renal transplantation. Our focus was to investigate whether fruit and vegetable intake is associated with long-term risk of PTDM in a cohort of stable RTRs.

RESEARCH DESIGN AND METHODS

Design and Study Population
For this study, we used data from a previously described cohort of 707 stable RTRs (15). All adult (≥18 years old) RTRs with a functioning allograft for ≥1 year after transplantation and who visited the outpatient clinic of the University Medical Center Groningen (UMCG; Groningen, the Netherlands) between November 2008 and March 2011 were invited to participate. RTRs with diabetes or a history of diabetes at baseline (n = 180) and nonresponders to dietary surveys (n = 55) were excluded, leaving 472 RTRs who were eligible for analysis. RTRs with missing dietary data did not materially differ from RTRs with complete data for age, sex, BMI, estimated glomerular filtration rate (eGFR), time between transplantation and study baseline, and PTDM incidence during follow-up. This study was conducted according to the guidelines in the Declaration of Helsinki, and the institutional review board approved the study protocol (METc 2008/186).

Renal Transplant Characteristics
RTRs all received transplants at the UMCG and were treated with standard antihypertensive and immunosuppressive therapy. Standard immunosuppression consisted of the following: azathioprine (100 mg/day) and prednisolone (starting with 20 mg/day and tapering to 10 mg/day) from 1968 to 1989; cyclosporine (target trough levels 175–200 mg/L in the first 3 months, 100 mg/L thereafter) and prednisolone (starting with 20 mg/day and tapering to 10 mg/day) from 1989 to 1996. In 1997 mycophenolate mofetil (2 g/day) was added to the standard immunosuppressive regimen, and for RTRs with no complications, cyclosporine was slowly withdrawn from 1 year after transplantation onward. In 2012 cyclosporine was replaced by tacrolimus, and RTRs continued triple-immunosuppressive therapy with prednisolone (20 mg/day, tapering to 5 mg/day), tacrolimus (target trough levels 8–12 μg/L in the first 3 months, 6–10 μg/L until month 6, and 4–6 μg/L from 6 months onward), and mycophenolate mofetil (starting with 2 g/day, tapering to 1 g/day). Upon hospital discharge after transplantation, all RTRs were visited by a dietitian from the UMCG. Except for discouraging excess sodium intake, describing the use of food-safety techniques, and encouraging weight loss in overweight individuals, no specific dietary counseling was performed. Adherence to dietary recommendations and changes in body weight since transplantation were evaluated 3 months after transplantation by a dietitian at the outpatient clinic of the UMCG and were recorded in patients' medical records.

Transplantation characteristics were retrieved from the local UMCG Renal Transplantation Database. Information on smoking behavior was obtained by using a questionnaire. Physical activity was assessed by using the Short Questionnaire to Assess Health-enhancing Physical Activity (SQUASH) score (time × intensity) (16).

Dietary Assessment
Dietary intake was assessed at baseline by using a validated food frequency questionnaire (FFQ) (17,18). Patients self-administered the questionnaire, and a trained researcher checked it for completeness on the day of the visit to the outpatient clinic. Inconsistent answers were verified with the patients. The FFQ inquired about consumption of 177 food items during the past month, taking seasonal variations into account, and included 7 fruit items and 18 vegetable items. Frequency was recorded in times per day, week, or month, and servings were expressed as natural units or household measures. The FFQ was linked to the Dutch Food Composition Table (NEVO) in order to calculate total energy intake (19).

Baseline Measurements
All measurements were performed once at baseline during a morning visit to the outpatient clinic. BMI was calculated as weight (kilograms) divided by height (meters squared). Waist circumference was measured on bare skin midway between the 10th rib and the iliac crest. Blood pressure and heart rate were measured by using a semiautomatic device (Dinamap 1846; Critikon, Tampa, FL). Blood was drawn in the morning after 8–12 h of fasting. Plasma glucose level was measured with a Roche P Analyzer, and HbA$_{1c}$ was assayed by turbidimetric inhibition immunoassay (Roche Diagnostics, Basel, Switzerland). Serum creatinine was measured by using an enzymatic isotope dilution assay, traceable on mass spectrometry, on a Roche P-Modulator automated analyzer (Roche Diagnostics, Basel, Switzerland), and renal function was assessed by using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula for creatinine in order to calculate eGFR (20). On the basis of the definition from the National Cholesterol Education Program Expert Panel, metabolic syndrome was defined as three or more of the following components being present, or medication being used to control these traits: 1) waist circumference >102 cm in men, >88 cm in women; 2) serum triglycerides ≥1.70 mmol/L; 3) HDL cholesterol <1.03 mmol/L in men, <1.29 mmol/L in women; 4) blood pressure ≥130/85 mmHg; 5) fasting plasma glucose ≥6.1 mmol/L (21).

PTDM
PTDM was diagnosed, according to the American Diabetes Association criteria, when at least one of the following criteria was met: 1) symptoms of diabetes (e.g., polyuria, polydipsia, unexplained weight

loss) plus a nonfasting plasma glucose concentration $\geq$11.1 mmol/L (200 mg/dL), *2*) fasting plasma glucose (FPG) $\geq$7.0 mmol/L (126 mg/dL), *3*) start of antidiabetes medication, or *4*) plasma HbA$_{1c}$ $\geq$6.5% (48 mmol/mol) (22,23). PTDM was recorded until 30 September 2015. RTRs were censored for PTDM at the time of graft failure (i.e., they returned to dialysis or received another transplantation) or death.

Statistical Analyses

Data were analyzed with SPSS version 22.0 (IBM Corp., Armonk, NY), R version 3.2.3 (The R Foundation for Statistical Computing, Vienna, Austria), and STATA version 13 (StataCorp LP, College Station, TX). Normally distributed data are expressed as the mean $\pm$ SD, whereas non–normally distributed data are expressed as median (interquartile range [IQR]). Nominal data are summarized as percentages. Associations between baseline characteristics and fruit and vegetable intake were analyzed through linear regression. Data with skewed distribution were log-transformed when appropriate.

In prospective analyses we used Cox regression models to analyze whether fruit and vegetable intake were associated with risk of PTDM. The associations of fruit and vegetable intake with risk of PTDM were analyzed both as continuous and categorical variables. In Cox regression analyses with continuous variables, fruit and vegetable intake were log-transformed to provide the best-fitting model with a log2 base in order to express the hazard ratio (HR) per doubled daily intake. For categorical analyses, subjects were divided according to tertiles of daily intake of both fruits and vegetables. Tests of linear trend for categorical analyses were conducted by assigning the medians of daily intakes in tertiles, treated as a continuous variable. To account for potential confounding, we performed multivariable Cox regression analyses in which we cumulatively adjusted for age, sex, time between transplantation and study baseline (model 1), eGFR, proteinuria (model 2), HbA$_{1c}$, and FPG (model 3). To avoid including too many variables for the number of events in Cox regression analyses, further models were created that performed additive adjustments to model 3. In additive multivariable models

we adjusted for SQUASH score, total energy intake, and BMI (model 4); smoking behavior and alcohol use (model 5); cytomegalovirus (CMV) infection, acute rejection, and hepatitis C virus (HCV) infection (model 6); prednisolone dose and tacrolimus use (model 7); and use of diuretics, β-blockers, and renin-angiotensin-aldosterone system inhibitors (model 8). We used penalized splines to visualize the association of fruit and vegetable intake on risk of PTDM by fitting Cox regression models according to model 3. Power calculations based on an assumption of 80% power and a two-sided α of 0.05 indicated that the minimum detectable HR was 0.78 for fruit intake and development of PTDM and 0.65 for vegetable intake and development of PTDM.

In further analyses, we investigated whether components of the metabolic syndrome serve as intermediate variables (mediators) in the association of vegetable intake and risk of PTDM. To establish mediation, we performed mediation analyses according to the method described by Preacher and Hayes, by which we tested statistical significance and magnitude of mediation (24) (see the Supplementary material for a detailed description of the procedure). In all analyses, a two-sided *P* value <0.05 was considered statistically significant.

RESULTS

Patient Characteristics

Baseline characteristics of the study population are shown in Table 1. RTRs included in the cohort were 51 $\pm$ 13 years old, 267 (56.6%) were male, and the majority (99.6%) was Caucasian. At study baseline, 266 RTRs received dual-immunosuppressive maintenance therapy, which included prednisolone and one other immunosuppressive drug (i.e., mycophenolate mofetil, azathioprine, cyclosporine, tacrolimus, or sirolimus); 195 RTRs received triple-immunosuppressive maintenance therapy that included prednisolone; 8 RTRs received mono-immunosuppressive maintenance therapy with prednisolone; and 3 RTRs received no prednisolone as part of their immunosuppressive regimen. Median fruit intake was 99 g/day (IQR 44–192 g/day), and median vegetable intake was 108 g/day (IQR 71–153 g/day). Univariable associations of baseline characteristics with fruit and vegetable intakes are shown in Table 1.

Fruit intake was positively associated with vegetable intake and age, and inversely with male sex, diastolic blood pressure, and current smoking. In age- and sex-adjusted multivariable linear regression analyses, vegetable intake and current smoking remained associated with fruit intake, while the association with diastolic blood pressure became nonsignificant. Vegetable intake was positively associated with fruit intake, HDL cholesterol, total energy intake, physical activity, and alcohol use, and inversely associated with BMI, waist circumference, diastolic blood pressure, proteinuria, LDL cholesterol, and fasting triglycerides. In age- and sex-adjusted multivariable linear regression analyses, all associations remained except the inverse associations with diastolic blood pressure and proteinuria. Baseline characteristics according to tertiles of fruit and vegetable intake are shown in Supplementary Tables 1 and 2, respectively.

Development of PTDM

During median follow-up for 5.2 years (IQR 4.1–5.8 years), 52 RTRs (11%) developed PTDM after a median of 2.3 years (IQR 1.2–4.0 years). PTDM was diagnosed in 2 RTRs on the basis of the presence of polyuria plus nonfasting glucose $\geq$11.1 mmol/L, in 8 RTRs on the basis of FPG $\geq$7.0 mmol/L, in 35 RTRs through HbA$_{1c}$ $\geq$6.5%, and in 7 RTRs because antidiabetes medication was initiated by other health care professionals. RTRs who developed PTDM were 52.7 $\pm$ 10.7 years old, 59.6% were male, BMI was 27.3 $\pm$ 4.5 kg/m^2, and waist circumference was 103 $\pm$ 14 cm. FPG was 5.4 $\pm$ 0.7 mmol/L, HbA$_{1c}$ was 6.1% (IQR 5.9–6.3%), HDL cholesterol was 1.2 mmol/L (IQR 1.0–1.4 mmol/L), LDL cholesterol was 3.2 $\pm$ 1.0 mmol/L, and total cholesterol was 5.3 $\pm$ 1.5 mmol/L. Median time between transplantation and study baseline for the 52 RTRs who developed PTDM was 5.2 years (IQR 1.4–12.9 years), and 65% used a calcineurin inhibitor at study baseline.

Fruit and Vegetable Intake and Development of PTDM

Analyses of the prospective association between fruit intake and development of PTDM are shown in Table 2. Fruit intake was not associated with risk of PTDM in univariable analysis (HR 0.90 [95% CI

Table 1—Baseline characteristics of and their associations with fruit and vegetable intake in 472 RTRs

	Total population	Fruit intake		Vegetable intake	
		St. β	P	St. β	P
Fruit intake, g/day	99 (44–192)	—	—	0.22	<0.001
Vegetable intake, g/day	108 (71–153)	0.22	<0.001	—	—
Demographics					
Age, years	51.4 ± 13.2	0.22	<0.001	0.08	0.07
Caucasian	470 (99.6)	0.004	0.93	0.03	0.53
Male sex	267 (56.6)	−0.11	0.02	−0.07	0.12
BMI, kg/m^2	26 ± 4.5	−0.01	0.85	−0.11	0.02
Waist circumference, cm	97 ± 14	−0.04	0.44	−0.14	0.003
Hemodynamics					
SBP, mmHg	135 ± 17	−0.03	0.54	−0.06	0.20
DBP, mmHg	83 ± 11	−0.12	0.01	−0.10	0.04
Heart rate, bpm	68 ± 12	0.002	0.97	−0.04	0.47
Antihypertensive drug use	407 (86.2)	0.001	0.97	0.002	0.97
Transplant demographics					
Time since transplantation, years	5.5 (2.0–12.4)	0.03	0.49	0.03	0.46
Preemptive	84 (17.8)	0.01	0.83	0.06	0.23
Living donor	170 (36.0)	0.06	0.22	0.02	0.62
CMV infection	115 (24.4)	−0.001	0.99	0.02	0.69
HCV infection	6 (1.3)	0.07	0.11	−0.002	0.98
Immunosuppressive treatment					
Tacrolimus	79 (16.7)	−0.03	0.49	−0.06	0.23
Cyclosporine	178 (37.7)	−0.03	0.52	−0.03	0.57
Azathioprine	88 (18.6)	0.05	0.34	0.09	0.06
Mycophenolic acid	312 (66.1)	0.02	0.64	0.03	0.54
Prednisolone	469 (99.4)	−0.03	0.52	−0.04	0.40
Prednisolone dose, mg/day	10 (7.5–10.0)	−0.03	0.57	−0.07	0.14
Renal function					
Serum creatinine, μmol/L	122 (99–158)	−0.08	0.10	−0.07	0.11
eGFR, mL/min	53.1 ± 20.2	−0.01	0.84	0.01	0.78
Proteinuria	94 (19.9)	−0.04	0.39	−0.10	0.03
Metabolic parameters					
Metabolic syndrome	255 (54.0)	0.01	0.87	−0.04	0.35
FPG, mmol/L	5.2 ± 0.6	0.01	0.82	0.01	0.92
HbA$_{1c}$, %	5.7 (5.4–5.9)	0.03	0.55	−0.06	0.20
Total cholesterol, mmol/L	5.1 ± 1.1	−0.07	0.15	−0.04	0.35
HDL cholesterol, mmol/L	1.3 (1.1–1.7)	0.05	0.32	0.14	0.002
LDL cholesterol, mmol/L	3.0 ± 0.9	−0.05	0.29	−0.10	0.03
Triglycerides, mmol/L	1.6 (1.2–2.2)	−0.07	0.11	−0.13	0.01
hs-CRP, mg/L	1.4 (0.6–3.8)	0.02	0.76	−0.08	0.11
Lifestyle parameters					
Energy intake, kcal/day	2,207 ± 648	−0.04	0.38	0.15	0.001
SQUASH, time*intensity	5,605 (2,885–8,648)	0.07	0.12	0.10	0.04
Smoker	62 (13.1)	−0.21	<0.001	−0.08	0.09
Alcohol consumer	324 (68.6)	0.06	0.19	0.14	0.002

Data are the mean ± SD, median (interquartile range), or n (%), unless otherwise indicated. Significance was tested by using linear regression analysis for continuous data and the χ^2 test for categorical data. Non–normally distributed data were log-transformed when appropriate.

0.79–1.03] per ^{2}log g/day; P = 0.13) or in multivariable analyses after adjusting for age, sex, time between transplantation and study baseline, eGFR, proteinuria, HbA$_{1c}$, and FPG (model 3: HR 0.96 [95% CI 0.82–1.12] per ^{2}log g/day; P = 0.16). Likewise, no associations with development of PTDM were observed when fruit intake was analyzed according to tertiles of intake (P$_{trend}$ = 0.32). A penalized spline of the association between fruit intake and risk of PTDM is shown in Fig. 1.

Analyses of the prospective association of vegetable intake with development of PTDM are depicted in Table 3. Vegetable intake was significantly associated with a lower risk of PTDM in a univariable analysis (HR 0.77 [95% CI 0.63–0.94] per ^{2}log g/day; P = 0.009). This association remained independent of age, sex, time between transplantation and study baseline, renal function, HbA$_{1c}$, and FPG (model 3: HR 0.76 [95% CI 0.62–0.92] per ^{2}log g/day; P = 0.005). Additional adjustment for other potential confounders—including physical activity, total energy intake, and BMI (model 4); smoking behavior and alcohol use (model 5); CMV infection, acute rejection, and HCV infection (model 6); prednisolone dose and tacrolimus use (model 7); and use of diuretics, β-blockers, and renin-angiotensin-aldosterone system inhibitors (model 8)—did not materially alter the association between vegetable intake and risk of PTDM. Similarly, in categorical analyses according to tertiles of vegetable intake, lower vegetable intake was significantly associated with increased risk of PTDM (P$_{trend}$ = 0.02). Moreover, compared with RTRs in the lowest tertile of vegetable intake (median intake 53 g/day [IQR 34–71 g/day]), RTRs in the highest tertile of vegetable intake (median intake 175 g/day [IQR 153–216 g/day]) had a more than 50% lower risk of PTDM (HR 0.45 [95% CI 0.23–0.90]; P = 0.02). Additional adjustment for potential confounders did not materially alter these associations. A penalized spline of the association between vegetable intake and risk of PTDM is shown in Fig. 1.

Additionally, we performed Cox regression analyses according to subcategories of vegetables (i.e., green legumes, cruciferous vegetables, leafy green vegetables, and other vegetables). In these analyses, both intake of cruciferous vegetables (HR 0.81 [95% CI 0.69–0.96] per ^{2}log g/day; P = 0.02) and leafy green vegetables (HR 0.70 [95% CI 0.53–0.93] per ^{2}log g/day; P = 0.01) were significantly associated with lower risk of PTDM, independent of potential confounders including age, sex, time between transplantation and study baseline, eGFR, proteinuria, HbA$_{1c}$, and FPG. Similar analyses for intake of green legumes and other vegetables showed that neither intake of green legumes (HR 0.91 [95% CI 0.78–1.07] per ^{2}log g/day; P = 0.24) nor intake of other vegetables (HR 0.92 [95% CI 0.78–1.08] per ^{2}log g/day; P = 0.31) were significantly associated with development of PTDM.

Table 2—Prospective survival analysis of fruit intake and PTDM

	Tertile 1	Tertile 2	Tertile 3		Continuous (per ^{2}log g/day)	
Median fruit intake, g/day	26 (11–44)	99 (83–123)	232 (192–246)		99 (44–192)	
RTRs, n	157	158	157		472	
Events, n	19	18	15		52	
Models*	Reference	HR (95% CI)	HR (95% CI)	P for trend	HR (95% CI)	P
Crude	1.00	0.90 (0.47–1.72)	0.71 (0.36–1.40)	0.32	0.90 (0.79–1.03)	0.13
1	1.00	0.86 (0.45–1.65)	0.65 (0.32–1.32)	0.23	0.89 (0.78–1.02)	0.09
2	1.00	0.88 (0.46–1.70)	0.67 (0.33–1.36)	0.26	0.89 (0.78–1.02)	0.10
3	1.00	0.84 (0.43–1.67)	0.77 (0.37–1.60)	0.50	0.90 (0.79–1.04)	0.16
4	1.00	0.96 (0.47–1.97)	0.85 (0.40–1.84)	0.68	0.92 (0.79–1.07)	0.28
5	1.00	0.82 (0.41–1.64)	0.86 (0.41–1.81)	0.72	0.92 (0.80–1.06)	0.26
6	1.00	0.84 (0.41–1.71)	0.84 (0.39–1.77)	0.67	0.91 (0.79–1.06)	0.21
7	1.00	0.88 (0.44–1.74)	0.81 (0.39–1.70)	0.59	0.92 (0.79–1.05)	0.22
8	1.00	0.95 (0.47–1.90)	0.85 (0.41–1.79)	0.67	0.91 (0.79–1.06)	0.22

*Model 1 included crude data and adjusted for age, sex, and the time between transplantation and study baseline. Model 2 adjusted for model 1 variables, eGFR, and proteinuria (>0.5 g/24 h). Model 3 adjusted for model 2 variables, HbA$_{1c}$, and FPG. Model 4 adjusted for model 3 variables, SQUASH score, total energy intake (kilocalories per day), and BMI. Model 5 adjusted for model 3 variables, smoking status, and alcohol use. Model 6 adjusted for model 3 variables, CMV infection, acute rejection, and HCV status. Model 7 adjusted for model 3 variables, the use of prednisolone and daily dose, and the use of tacrolimus. Model 8 adjusted for model 3 variables, the use of RAAS inhibitors, the use of β-blockers, and the use of diuretics.
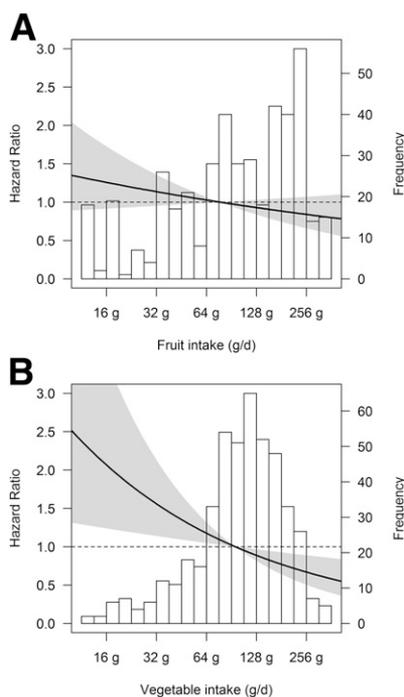
Mediation Analyses

Blood pressure and FPG were not independently associated with vegetable intake and therefore were eliminated as

Figure 1—Associations of fruit intake (A) and vegetable intake (B) with risk of PTDM. Data were fit by a Cox proportional hazard regression model based on a penalized spline and adjusted for age, sex, time after transplantation, eGFR, proteinuria, HbA$_{1c}$, and FPG. Data are shown on a ^{2}log scale. The gray areas represent the 95% CI.

potential mediators in the association between vegetable intake and PTDM. Otherwise, HDL cholesterol, triglycerides, and waist circumference were all independently significantly associated with vegetable intake and PTDM. Therefore HDL cholesterol, triglycerides, and waist circumference were considered as potential mediators in the association between vegetable intake and PTDM, because associations between higher vegetable intake and these components of the metabolic syndrome are more likely to be a consequence of high vegetable intake than the inverse, which would imply that a high vegetable intake is the consequence of not having the metabolic syndrome. Results of mediation analyses with components of the metabolic syndrome are shown in Supplementary Table 3. In mediation analyses, HDL cholesterol, triglycerides, and waist circumference mediated 28.3, 26.5, and 20.9% of the inverse association between vegetable intake and risk of PTDM, respectively. Conjointly, HDL cholesterol, triglycerides, and waist circumference mediated 49.7% of the association of vegetable intake and risk of PTDM.

CONCLUSIONS

In this prospective study, we investigated the prospective associations of fruit and vegetable intake with development of PTDM in a large cohort of stable RTRs. We found that low vegetable intake was

independently associated with higher risk of PTDM after renal transplantation. Furthermore, we show that the inverse association of vegetable intake and risk of PTDM was mediated to a considerable extent (49.7%) by higher HDL cholesterol levels, lower triglyceride levels, and smaller waist circumference, all of which are associated with—and likely at least partly effects of—higher vegetable intake.

In our cohort of RTRs, median fruit and vegetable intakes were far below the recommended intake of 200 g/day for each. These findings are consistent with estimates of the general Dutch population, as reported in the Dutch National Food Consumption Survey 2007–2010 (25). However, median intakes of fruits and vegetables in our study were lower than those reported among the general Dutch population. Most patients with end-stage renal disease are advised to consume a low-protein, low-potassium, low-sodium diet, which also often restricts fluid intake. This diet prevents excessive urea generation and development of hyperphosphatemia, hyperkalemia, and hypertension. Consequently, these dietary restrictions limit intakes of fruits and vegetables, which are rich sources of potassium. Low fruit and vegetable intakes have also been reported in patients who undergo dialysis treatment; these low intakes are most likely the result of dietary restrictions discouraging potassium intake in order to prevent hyperkalemia (26,27).

Table 3—Prospective survival analysis of vegetable intake and PTDM

	Tertile 1	Tertile 2	Tertile 3		Continuous (per ^{2}log g/day)	
Median vegetable intake, g/day	53 (34–71)	108 (93–119)	175 (153–216)		108 (71–153)	
RTRs, n	157	158	157		472	
Events, n	24	16	12		52	
Models*	Reference	HR (95% CI)	HR (95% CI)	P for trend	HR (95% CI)	P
Crude	1.00	0.60 (0.32–1.12)	0.45 (0.23–0.90)	0.02	0.77 (0.63–0.94)	0.009
1	1.00	0.61 (0.32–1.16)	0.43 (0.21–0.86)	0.02	0.76 (0.63–0.93)	0.007
2	1.00	0.62 (0.33–1.18)	0.44 (0.22–0.88)	0.02	0.77 (0.63–0.94)	0.009
3	1.00	0.54 (0.28–1.02)	0.39 (0.19–0.80)	0.009	0.76 (0.62–0.92)	0.005
4	1.00	0.55 (0.28–1.07)	0.40 (0.19–0.86)	0.02	0.78 (0.63–0.98)	0.03
5	1.00	0.56 (0.29–1.07)	0.43 (0.20–0.92)	0.03	0.75 (0.60–0.94)	0.01
6	1.00	0.49 (0.25–0.95)	0.37 (0.18–0.79)	0.009	0.74 (0.60–0.91)	0.004
7	1.00	0.56 (0.29–1.06)	0.41 (0.20–0.86)	0.02	0.77 (0.63–0.94)	0.01
8	1.00	0.56 (0.29–1.07)	0.44 (0.21–0.91)	0.02	0.79 (0.64–0.97)	0.02

*Model 1 includes the crude data adjusted for age, sex, and time between transplantation and study baseline. Model 2 adjusts for model 1 variables, eGFR, and proteinuria (>0.5 g/24 h). Model 3 adjusts for model 2 variables, HbA$_{1c}$, and FPG. Model 4 adjusts for model 3 variables, SQUASH score, total energy intake (kilocalories per day), and BMI. Model 5 adjusts for model 3 variables, smoking status, and alcohol use. Model 6 adjusts for model 3 variables, CMV infection, acute rejection, and HCV. Model 7 adjusts for model 3 variables, the use of prednisolone and daily dose, and the use of tacrolimus. Model 8 adjusts for model 3 variables, the use of RAAS inhibitors, the use of β-blockers, and the use of diuretics.

One might assume that the lower intakes of fruits and vegetables we noted could be a result of persisting adherence to these dietary restrictions imposed during the pretransplantation stage in order to limit potassium intake.

We did not observe a significant association of fruit intake with development of PTDM. Fruit intake has been described to reduce the risk of diabetes in the general population (28,29). It might be that in our study variation of fruit intake was too low or too few events of PTDM occurred, or a combination of both, for us to observe an otherwise existing association. Alternatively, it might be that RTRs are particularly susceptible to the adverse effects of fructose, which is present in large amounts in fruits and may predispose patients to weight gain and the metabolic syndrome (30). We did observe an inverse association of vegetable intake and risk of PTDM. The exact biological mechanisms responsible for the protective effect of vegetable intake against the development of diabetes remain largely unknown, although several mechanisms have been proposed. The protective effect of vegetable intake against diabetes might result from a higher intake of antioxidants, such as β-carotene and vitamins C and E, which are present in most vegetables. Additionally, fibers in vegetables might also contribute to the protective effect of vegetable intake against the risk of diabetes. High dietary fiber intake has been associated with a low risk of diabetes (31), which is thought to be due to improved satiety and lower energy intake, as well as improved postprandial hyperglycemia and insulin sensitivity as a result of slower absorption of nutrients and fiber fermentation in the colon (32). Furthermore, considering that vegetables are rich dietary sources of magnesium, the beneficial effect of vegetable intake on the risk of diabetes might also be attributed to higher magnesium intake. Recent meta-analyses show that low magnesium intake is associated with incidence of type 2 diabetes in the general population (33), and magnesium-supplementation has improved glucose hemostasis in both subjects with diabetes (34,35) and those without diabetes (36,37).

Additionally, we found that vegetable intake was significantly associated with components of the metabolic syndrome (i.e., HDL cholesterol, triglycerides, and waist circumference), and mediation analyses revealed that these components of the metabolic syndrome mediated to a considerable extent the inverse association of vegetable intake and risk for PTDM. In RTRs, the prevalence of the metabolic syndrome is high and has been associated with an increased risk for PTDM (38). Although data are limited regarding the relation between diet and the metabolic syndrome in RTRs, fruit and vegetable intake has been shown to reduce the risk of the metabolic syndrome in the general population (39).

Furthermore, vegetable intake has been inversely associated with weight gain during the 1st year after renal transplantation (40), a hallmark of the development of the metabolic syndrome (41,42). Our results suggest that increasing vegetable intake may reduce the risk of PTDM in this population, at least to a considerable extent, by improving the metabolic profile. Future studies aimed at increasing vegetable intake in RTRs are necessary in order to further substantiate these findings and elucidate the mechanisms underlying these associations.

Strengths of this study include a median follow-up of 5.2 years and PTDM as a clinically relevant end point. Furthermore, we included a large number of RTRs with complete dietary data and did not lose participants during follow-up. Moreover, we included only stable RTRs who had a functioning renal transplant for more than 1 year, thereby excluding transient posttransplantation hyperglycemia in the diagnosis of PTDM. On the other hand, RTRs in this study were included at a median of 5.5 years after transplantation, and 180 RTRs with diabetes at baseline were excluded from analyses. Therefore, caution should be taken to extrapolate the current results to RTRs in the early stages after transplantation. Also, no oral glucose tolerance tests were performed in this study, and this may have led to underdetection of PTDM. Furthermore, the incidence rate of PTDM in our study should be

interpreted in light of the prednisolone doses, which were relatively high compared with current immunosuppressive regimens; this might also contribute to the development of PTDM. Another limitation of this study is that dietary intake was assessed by using a self-reported FFQ, which might lead to over- or underreporting of dietary intake. Also, the FFQ was validated on the basis of serum lipids and protein intake but not fruit and vegetable intakes. Furthermore, this is a single-center study, and subjects are predominantly Caucasian, which hinders the extrapolation of our results to other populations, particularly because results from epidemiological studies investigating the association of vegetable intake with type 2 diabetes in non-Caucasian populations are scarce and show inconsistent results (43,44). Finally, the observational design of the study precludes drawing conclusions regarding causality.

In conclusion, in this study we found that vegetable intake, but not fruit intake, was associated with a lower long-term risk of PTDM in stable RTRs. Mediation analyses reveal that several components of the metabolic syndrome (i.e., HDL cholesterol, triglycerides, and waist circumference) substantially mediated the association between vegetable intake and risk of PTDM. Our findings further support current evidence that pleads for dietary recommendations to increase the daily intake of vegetables in RTRs.

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