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Urinary urea excretion and long-term outcome after renal transplantation

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Abstract

Background. Little is known about optimal protein intake after transplantation. The aim of this study was to prospectively investigate associations of urinary urea excretion, a marker for protein intake, with graft failure and mortality in renal transplant recipients (RTR) and potential effect-modification by body mass index (BMI) and estimated glomerular filtration rate (eGFR).

Methods. Urinary urea excretion was measured in repeated 24-hr urine collections between 6 and 18 months after transplantation.

Results. In total, 940 RTR were included. During 4.4 (2.3–7.8) years of follow-up for graft failure and 4.8 (2.5–8.3) years for all-cause mortality, 78 RTR developed graft failure and 158 RTR died. Urinary urea excretion was not associated with graft failure in the overall population, but was inversely associated with graft failure in RTR with a BMI less than 25 kg/m^2 (Hazard Ratio [HR], 0.64 [0.28–1.50] and 0.27 [0.09–0.83] for the second and third tertiles, respectively; $P < 0.001$), and in RTR with an eGFR of 45 mL per min per 1.73 m^2 or higher (HR, 0.34 [0.15–0.79]; $P = 0.015$ and HR, 0.31 [0.11–0.86]; $P = 0.025$ for the second and third tertiles, respectively), both independent of potential confounders. Compared to the first tertile, RTR in the second and third tertiles of urinary urea excretion were at a lower risk of all-cause mortality (HR, 0.47 [0.32–0.69]; $P < 0.001$ and HR, 0.42 [0.26–0.68]; $P < 0.001$, respectively), independent of potential confounders. Body mass index and eGFR did not influence this association.

Conclusions. Urinary urea excretion, a marker for protein intake, was inversely related to graft failure in RTR with a BMI less than 25 kg/m^2 and in RTR with an eGFR of 45 mL per min per 1.73 m^2 or higher. In addition, urinary urea excretion was inversely related to mortality.

Introduction

Optimizing protein intake is an important component of dietary management of chronic kidney disease (CKD) patients. Because high protein intake is supposed to aggravate proteinuria (1–4), scientific societies consider a low protein diet cornerstone of renoprotective treatment (3,5,6). Guidelines recommend daily allowances of 0.6 to 0.8 g protein/kg per day in patients with CKD stages 1 to 4 (3,5,6).

In patients with advanced CKD (stage 5), the opposite is true. Mild anorexia and low protein intake are often already present at CKD stage 3, but are particularly pronounced once patients enter dialysis (7–9). Hence, dialysis patients usually start with relatively low protein intake (10,11), while dialysis induces protein catabolism and losses that must be compensated. Low protein intake has been associated with lower survival rates in hemodialysis patients (12,13). Therefore, current guidelines recommend a minimal protein intake of 1.1 g/kg per day and preferably 1.2 to 1.3 g/kg per day in dialysis patients (6,14,15).

Although recommended intake is unambiguous for patients with CKD stage 1 to 4 and CKD stage 5 treated with or without dialysis, advisable protein intake is unknown after transplantation. Therefore, the aim of the present study was to prospectively investigate whether urinary urea excretion (UUE), a marker of protein intake, is associated with graft failure and all-cause mortality in renal transplant recipients (RTR).

Patients and methods

Study design and population

RTR were eligible for this study if they received a renal transplant for the first time between January 1993 and February 2008 in the University Medical Center Groningen in the Netherlands. Details of the cohort have been published previously (16). We made use of the regular patient care program for outpatients. For each visit, RTR are requested to collect a 24-hr urine sample in which several parameters including UUE, protein and sodium are routinely assessed. Data of total cholesterol, albumin, creatinine and 24-hr urinary excretion of urea, protein, sodium, and creatinine, assessed between 6 and 18 months, were extracted from our hospital laboratory system (Supplementary Figure S1). The median of these measurements was used for analyses. Patient characteristics were retrieved from medical records. To assess change in renal function over time, the difference between serum creatinine at baseline and last serum creatinine available was calculated. In case of graft failure or mortality, the last measurement before the event was used. Body surface area (BSA) was calculated as: $BSA = (W^{0.425} \times H^{0.725}) \times 0.007184$

(17) and body mass index (BMI) as weight (kg) by height (m) squared. Delta BMI was calculated by subtracting baseline BMI from the BMI at the time of transplantation. Delayed graft function was defined as oliguria for more than 6 days after transplantation.

Shortly after transplantation, RTR are often not yet stable, and infections and rejections play a major role. Therefore, data of the first 6 months after transplantation were discarded. Furthermore, we only included patients who survived with a functioning graft beyond 6 months after transplantation and beyond the period of baseline data collection. Finally, patients with missing data on UUE were excluded ($n = 9$, 0.84%), leaving 940 patients eligible for analyses.

The Institutional Review Board of the University Medical Center Groningen approved the study protocol, which adhered to the declaration of Helsinki. According to Dutch law, general consent for organ donation and transplantation includes consent for research projects.

Laboratory measurements

Non-fasting blood and urine samples were directly analyzed after collection. Measurements were performed on a Merck Mega Analyzer before March 2006 (Merck, Darmstadt, Germany). From March 2006 onward, measurements were performed on a Roche Modular (Roche Ltd., Mannheim, Germany). In the case of significant differences between results obtained by the Merck Mega and Roche Modular, results were converted according to conversion equations provided by our laboratory (Supplementary Table S1). Urea was measured with a urease glutamate dehydrogenase reaction (Intra-assay coefficient of variation Merck Mega 3.1% and Roche Modular 2.4%; Merck Mega and Roche Modular). Urinary urea excretion was based on a median (interquartile range) of 6 (5–8) collections of 24-hr urine per patient.

Clinical end-points

The primary end-point of this study was graft failure and the secondary endpoint was all-cause mortality. Graft failure was defined as return to dialysis or the need for retransplantation. Follow-up was recorded until October 2009. Person-time of follow-up was computed for each participant from 1.5 years after transplantation until the incidence of graft failure or death, or censored after 10 years of follow-up. There was no loss to follow-up.

Statistical analysis

Baseline characteristics of the study population were calculated. *P*-values for a linear trend across tertiles of UUE were determined with linear regression. The difference in decline of serum creatinine between tertiles of UUE was determined with a Mann Whitney U test. The estimated GFR (eGFR) was calculated using the CKD Epidemiology Collaboration equation (18).

Several patients had missing values for one or more variables. Bias is introduced if subjects with missing values are excluded. Therefore, we performed multiple imputation for values with less than 30% missing data. Finally, the results were combined into pooled results which were only used in Cox regression models.

Univariable survival analyses were performed using log-rank tests. Because sex is an important confounder in many associations (19), we first determined whether associations of UUE with graft failure and all-cause mortality were modified by sex. Furthermore, to discern whether there might be a parallel with the obesity paradox in dialysis patients (20), we explored potential effect modification by BMI. In addition, we investigated potential effect modification by eGFR. Separate Cox regression analyses were performed for men, for women, for RTR with a BMI less than 25 kg/m^2, and RTR with a BMI of 25 kg/m^2 or higher, for RTR with an eGFR less than 45 mL per min per 1.73 m^2 and for RTR with an eGFR of 45 mL per min per 1.73 m^2 or higher. Because similar results were obtained in men and women, results were combined for both sexes. Cumulative adjustment was performed in Cox regression analyses for age, sex, donor age and sex, urinary sodium excretion, eGFR, and BMI.

Furthermore, we estimated protein intake from UUE. Therefore, we used data from a previous cohort of RTR for which UUE and data on protein intake estimated by a food frequency questionnaire was available (21). Multivariable linear regression provided the following equations: protein intake in women (g/24 hr): $52.67 + 1.03 \times$ UUE (g/24 hr) $+ 0.10 \times$ age (years) - 3.30; protein intake in men (g/24 hr): $52.67 + 1.03 \times$ UUE (g/24 hr) $+ 0.10 \times$ age (years). Finally, Cox regression analyses were carried out for the association of estimated protein intake with graft failure and all-cause mortality.

To explore whether 24-hr UCE and serum albumin could lie in the causal pathway between UUE and outcome, we performed secondary analyses, in which we additionally adjusted for serum albumin and UCE. As further secondary analyses, we also repeated Cox regression analyses with adjustment for the change in BMI over the first posttransplant year rather than baseline BMI. Finally, to determine whether the use of high doses of corticosteroids (i.e. during acute rejection), might have influenced the observations, we performed secondary analyses in which Cox regression analyses were repeated after exclusion of 32 subjects who experienced an acute rejection between 5 and 18 months after transplantation.

Statistical analyses were performed using SPSS (version 20.0, IBM, Chicago, IL, USA) and STATA (version 11.0, StataCorp LP, College Station, Texas, USA). A *P*-value less than 0.05 was considered statistically significant.

Results

Patient characteristics

Median age was 50 (interquartile range, 40–59) years and 59% was male. Mean eGFR was 54.1 ± 18.3 mL per min per 1.73 m^2. Mean UUE was higher in men (24.3 ± 5.9 g/24 hr) compared to women (20.6 ± 5.4 g/24 hr; $P < 0.001$). Baseline characteristics are shown according to tertiles of UUE (Table 1). Prevalence of male sex, age, BSA, BMI, delta BMI, diastolic blood pressure, prevalence of diabetes, albumin, urinary sodium excretion, urinary creatinine excretion (UCE), delayed graft function, and use of proliferation inhibitors were positively associated with UUE. Prevalence of tubular interstitial disease as primary renal disease, use of mammalian target of rapamycin inhibitors and cold ischemic time were inversely related to UUE. Renal transplant recipients with greater UUE received a kidney more frequently from a living donor and less frequently from a CMV seropositive donor. Urinary urea excretion was not significantly associated with eGFR, urinary protein excretion, and maintenance dose of corticosteroids.

Table 1. Baseline characteristics according to tertiles of urea excretion in 940 renal transplant recipients.

		Tertiles of urinary urea excretion g/24 hr[b]			
	N[a]	I	II	III	*P*[c]
Urinary urea, g/24 hr	940	16.6 ± 2.5	22.3 ± 1.4	29.4 ± 4.0	
Recipient demographics					
Age, yr	940	49 (37–60)	52 (39–59)	50 (42–59)	0.031
Men, n (%)	940	126 (40)	191 (61)	234 (75)	< 0.001
BSA, m^2	910	1.83 ± 0.16	1.93 ± 0.17	2.05 ± 0.20	< 0.001
BMI, kg/m^2	910	25 ± 4	26 ± 4	28 ± 5	< 0.001
Delta BMI[d]	696	1.5 ± 2.5	1.6 ± 2.6	1.8 ± 2.4	0.031
Blood pressure					
Systolic, mm Hg	899	143 (130–155)	140 (132–153)	143 (133–153)	0.31
Diastolic, mm Hg	899	83 (77–90)	83 (79–90)	85 (79–91)	0.024
Total cholesterol, mg/dL	866	223 ± 47	218 ± 41	214 ± 38	0.071
Diabetes, n (%)	732	15 (5)	26 (8)	31 (10)	0.013
Primary renal disease, n (%)	916				
Primary glomerular disease		67 (21)	81 (26)	88 (28)	0.052
Glomerulonephritis		14 (5)	24 (8)	16 (5)	0.75
Tubular interstitial disease		42 (13)	32 (10)	24 (8)	0.004
Polycystic renal disease		57 (18)	53 (17)	63 (20)	0.18
Dysplasia and hypoplasia		9 (3)	5 (2)	3 (1)	0.091
Renovascular disease		22 (7)	23 (7)	32 (10)	0.42

Table 1. (continued)

		Tertiles of urinary urea excretion g/24 hr[b]			
	N[a]	I	II	III	*P*[c]
Diabetic nephropathy		11 (4)	14 (5)	14 (5)	0.92
Other or unknown cause		82 (26)	71 (23)	69 (22)	0.58
Donor demographics					
Donor age, yr	940	46 (35–54)	47 (34–55)	47 (35–55)	0.73
Male donor, n (%)	940	161 (51)	162 (52)	152 (49)	0.65
Renal allograft function					
Serum albumin, g/dL	940	4.2 (4.0–4.4)	4.2 (4.1–4.4)	4.2 (4.1–4.4)	0.007
Serum creatinine, mg/dL	940	1.35 (1.08–1.82)	1.37 (1.12–1.70)	1.41 (1.21–1.75)	0.39
eGFR, ml/min/1.73m^2	940	52.7 ± 18.8	55.1 ± 18.6	54.4 ± 17.5	0.14
Urinary protein, g/24 hr	940	0.20 (0.11–0.32)	0.20 (0.11–0.30)	0.25 (0.14–0.39)	0.15
Urinary sodium, mEq/24 hr	940	129 ± 37	154 ± 42	191 ± 55	<0.001
Urinary creatinine, mg/24 hr	940	1149 ± 245	1373 ± 275	1633 ± 323	<0.001
Transplantation details					
Living donor, n (%)	940	63 (20)	86 (27)	100 (32)	<0.001
Acute rejection, n (%)	940	100 (32)	98 (31)	116 (37)	0.38
Delayed graft function, n (%)	940	77 (25)	71 (23)	99 (32)	0.040
HLA mismatches, n	746	2 (0–3)	2 (1–3)	2 (1–3)	0.51
Cold ischemic time, hr	939	18 (13–24)	17 (3–23)	16 (3–21)	<0.001
Warm ischemia time, min	940	41 ± 12	40 ± 13	42 ± 13	0.43
Dialysis duration prior to Tx, mo	919	37 (18–55)	35 (17–57)	37 (18–58)	0.64
CMV status					
CMV seropositivity recipient, n (%)	661	133 (43)	117 (37)	116 (37)	0.52
CMV seropositivity donor, n (%)	935	155 (50)	164 (52)	143 (46)	0.044
Immunosuppressants					
Use of corticosteroids, n (%)	940	303 (97)	305 (97)	301 (96)	0.59
Maintenance dose, mg/day	776	10 (10–10)	10 (10–10)	10 (10–10)	0.26
Use of calcineurin inhibitor, n (%)	940	267 (88)	277 (88)	281 (90)	0.28
Use of proliferation inhibitor, n (%)	940	232 (74)	243 (77)	260 (83)	0.007
Use of mTOR, n (%)	940	15 (5)	12 (4)	8 (3)	0.027
Other immunosuppressants, n (%)	940	68 (22)	66 (21)	80 (26)	0.06

[a]Number of patients with complete data. [b]Range of urinary urea excretion (g/24 hr) in tertiles: I: ≤20.1, II: 20.1–24.8, III: ≥ 24.8. [c]P for linear trend is shown. [d]Delta BMI was calculated as the difference between baseline BMI and BMI at transplantation. Dialysis duration prior to transplantation, urinary protein, and serum creatinine were log transformed. Normally distributed data are given as mean ± standard deviation (SD), skewed data are presented as median (interquartile range), and categorical distributed variables are given as number (percentage). SI conversion factors: To convert urinary urea from g/24 hr to mmol/24 hr, multiply values by 16.6513; total cholesterol from mg/dL to mmol/L, multiply values by 0.02586; serum creatinine from mg/dL to μmol/L , multiply values by 88.4; urinary creatinine from mg/24 hr to mmol/24 hr, multiply values by 0.00884. BMI, body mass index; BSA, body surface area; eGFR, estimated glomerular filtration rate; Tx, transplantation; CMV, cytomegalovirus; mTOR, mammalian target of rapamycin; IQR, interquartile range.

UUE and graft failure

Decline in serum creatinine was least pronounced in RTR in the second tertile of UUE ($P = 0.048$, compared to the first tertile). Serum creatinine was not different between the first and third tertile of UUE ($P = 0.34$). In line with this observation, graft failure rates were 36 (12%) in the first, 19 (6%) in the second, and 23 (7%) in the third tertile of UUE (log-rank; $P = 0.041$; Figure 1). In Cox regression analyses, UUE was not associated with graft failure, when all RTR were analyzed together (Table 2). However, there was an interaction with BMI. In RTR with a BMI less than 25 kg/m^2, we observed a strong inverse association of UUE with graft failure (Supplementary Table S2). Urinary urea excretion was not associated with graft failure in RTR with a BMI of 25 kg/m^2 or higher (Supplementary Table S3). Furthermore, UUE was not associated with graft failure in RTR with an eGFR less than 45 mL per min per 1.73 m^2 (Supplementary Table S4). However, there was an independent inverse association of UUE and graft failure in RTR with an eGFR of 45 mL per min per 1.73 m^2 or higher (Supplementary Table S5).

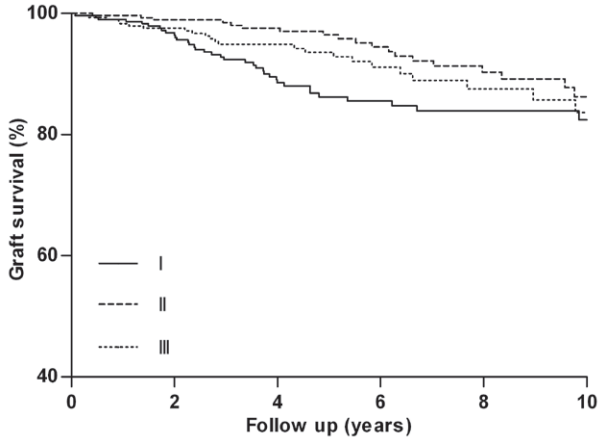

Figure 1. Kaplan Meier curves for graft failure according to tertiles of urinary urea excretion. Log-rank test for graft failure; $P = 0.041$. Range of urinary urea excretion (g/24 hr) in tertiles: I: ≤20.1, II: 20.1–24.8, III: ≥ 24.8.

UUE and all-cause mortality

During 4.8 (2.5–8.3) years of follow-up, 158 RTR died. All-cause mortality rates were twice as low in the second and third tertiles of UUE (Table 3 and Figure 2). This association was independent of sex, BMI, eGFR and other potential confounders.

Because the first tertile of UUE was associated with a greater risk of mortality compared to the second and third tertiles, we estimated the protein intake for the upper border of the first tertile. This upper border was 20.1 g per 24 hr. According to our equa-

tion (*Materials and Methods*), for a 50-year-old man with a weight of 84 kg, this translates into an estimated protein intake of 0.93 g/kg per day. In a woman of the same age, with a weight of 74 kg, this corresponds to an estimated protein intake of 1.01 g/kg per day.

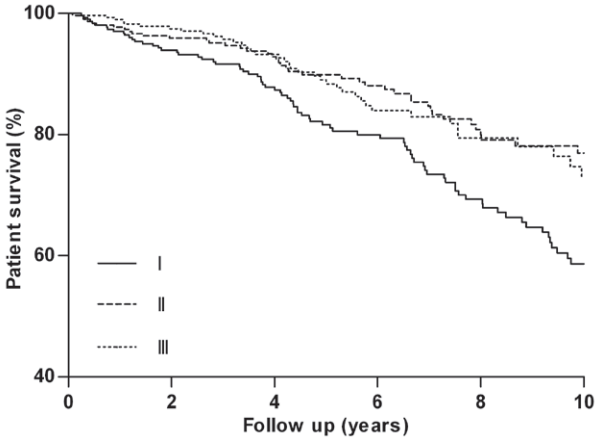

Figure 2. Kaplan Meier curves for mortality according to tertiles of urinary urea excretion. Log-rank test for mortality; $P = 0.001$. Range of urinary urea excretion (g/24 hr) in tertiles: I: ≤20.1, II: 20.1–24.8, III: ≥ 24.8.

Table 2. Cox regression analyses for graft failure according to tertiles of urinary urea excretion.

	Tertiles of urinary urea excretion[a]				
	I	II		III	
	Reference category	HR (95% CI)	***P***	HR (95% CI)	***P***
No. events	36	19		23	
Model 1	1.0	0.50 (0.29–0.87)	0.014	0.71 (0.42–1.19)	0.19
Model 2	1.0	0.49 (0.28–0.86)	0.013	0.69 (0.40–1.20)	0.19
Model 3	1.0	0.49 (0.28–0.87)	0.015	0.70 (0.40–1.21)	0.20
Model 4	1.0	0.47 (0.26–0.84)	0.012	0.66 (0.34–1.26)	0.21

[a]*Range of urinary urea excretion (g/24 hr) in tertiles: I: ≤20.1, II: 20.1–24.8, III: ≥ 24.8.*

Model 1: Crude association

Model 2: Model 1 + age, sex

Model 3: Model 2 + donor age, and donor sex

Model 4: Model 3 + urinary sodium excretion, eGFR, and BMI

HR, hazard ratio; CI, confidence interval; eGFR, estimated glomerular filtration rate; BMI, body mass index

Table 3. Cox regression analyses for mortality according to tertiles of urinary urea excretion.

	Tertiles of urinary urea excretion[a]				
	I	II		III	
	Reference category	HR (95% CI)	*P*	HR (95% CI)	*P*
No. events	78	42		38	
Model 1	1.0	0.54 (0.37–0.78)	0.001	0.59 (0.40–0.86)	0.007
Model 2	1.0	0.48 (0.33–0.70)	< 0.001	0.52 (0.35–0.78)	0.002
Model 3	1.0	0.48 (0.33–0.71)	< 0.001	0.53 (0.35–0.79)	0.002
Model 4	1.0	0.47 (0.32–0.69)	< 0.001	0.42 (0.26–0.68)	< 0.001

[a]*Range of urinary urea excretion (g/24 hr) in tertiles: I: ≤20.1, II: 20.1–24.8, III: ≥ 24.8.*

Model 1: Crude association

Model 2: Model 1 + age, sex

Model 3: Model 2 + donor age, and donor sex

Model 4: Model 3 + urinary sodium excretion, eGFR, and BMI

HR, hazard ratio; CI, confidence interval; eGFR, estimated glomerular filtration rate; BMI, body mass index

Secondary analyses

In secondary analyses, we repeated Cox regression analyses with estimated protein intake, based on our equation, instead of UUE. In these analyses, results remained essentially unchanged. Furthermore, results remained essentially unchanged after additional adjustment for serum albumin for graft failure and mortality. However, adjustment for UCE caused marked weakening of associations of UUE with graft failure (Hazard Ratio [HR], 0.59 [0.32–1.09]; $P = 0.092$ and HR, 1.02 [0.52–2.00]; $P = 0.95$ in the second and third tertiles, respectively) and mortality (HR, 0.61 [0.41–0.91]; $P = 0.016$ and HR, 0.62 [0.89–0.55]; $P = 0.62$ in the second and third tertiles, respectively). In addition, Cox regression analyses were repeated with adjustment for the change in BMI over the first post-transplant year rather than baseline BMI. Results were materially similar for graft failure and mortality. Furthermore, similar results were obtained for graft failure and mortality after exclusion of subjects who experienced rejection during the baseline assessment period (n = 32).

Discussion

UUE was strongly inversely associated with graft failure in RTR with a BMI less than 25 kg/m^2 and in RTR with an eGFR of 45 mL per min per 1.73m^2 or higher. Urinary urea excretion was not related to graft failure in RTR with a BMI of 25 kg/m^2 or higher or in RTR with an eGFR less than 45 mL per min per 1.73m^2. A more than 50% lower all-cause mortality risk was found in the second and third tertiles of UUE. This association was independent of BMI.

To our knowledge, this study is the first to prospectively investigate associations of protein intake, assessed by UUE, with late graft failure and mortality in a large cohort of RTR. Another method to assess protein intake is by means of a food frequency questionnaire (FFQ). In one cross-sectional study in RTR using an FFQ, no association was found of protein intake with renal function (21).

A study of Dunkler et al. (17) showed that FFQ-derived protein intake was inversely associated with CKD in patients with type 2 diabetes. In contrast, two reports of the modification of diet in renal disease (MDRD) intervention study (22,23) showed that neither a low (0.58 g/kg/d) nor a very low (0.28 g/kg/d) protein diet slowed renal function progression (22,23), but the assignment to a very low protein diet increased the risk of death in subjects with CKD stage 4 (23). In line, we also found no association of UUE with graft failure in the overall population or in RTR with an eGFR less than 45 mL per min per 1.73 m^2, but RTR with a relatively preserved renal function showed a slightly beneficial effect of protein intake on graft failure.

Alongside an FFQ, protein intake can be estimated with the equation by Maroni et al. which is based on UUE. The equation of Maroni et al. was used in a prospective study investigating the association of estimated protein intake and renal function decline in 48 RTR (24). RTR were stratified into a low or high protein group. Renal function deteriorated most in the high protein group. Unfortunately, in that study associations of estimated protein intake with graft failure and mortality were not studied. Our results of an inverse association of protein intake with mortality are in accordance with a study by Halbesma et al. (25) which showed that healthy subjects with the lowest estimated protein intake had the highest all-cause mortality rates.

In our population of RTR, optimal protein intake was higher than what is currently recommended in CKD patients (0.6–0.8 g/kg/day) (3,5,6), but comparable to recommended protein intake in patients treated with dialysis (≥ 1.1 g/kg/day) (6,14,15). Possibly, corticosteroid treatment in RTR induced a tendency for protein catabolism, hereby shifting the optimal protein intake upward. We acknowledge that in the current transplant era, many RTR are transplanted steroid free. However, RTR not using steroids are at an increased risk of acute rejection and chronic allograft nephropathy long-term after transplantation (26,27). Hence, within the first year after transplantation, steroids

are often added to a treatment regimen that was initially free of corticosteroids (26), resulting in steroids still being part of the long-term maintenance treatment regimen in approximately 70% of cases (26). We, therefore, believe that our data are applicable to many outpatient RTR long-term after transplantation.

Low protein intake may result in decreased muscle mass. Low UCE, as a marker of low muscle mass, has been consistently shown to be a predictor of mortality in the general population (28) and RTR (29–31). Hence, muscle mass hypothetically lies in the causal pathway between protein intake and outcome. Adjustment for variables in the causal pathway between exposure and outcome may induce bias and can cause significant associations to disappear (32). Indeed, adjustment for UCE caused material weakening of associations of UUE with graft failure and mortality, suggesting that UCE reflecting muscle mass indeed lies in the causal pathway between UUE reflecting protein intake and outcome.

In dialysis patients, robust inverse associations were found of BMI with mortality rates (20,33). This phenomenon is called the 'obesity paradox' (20,33). To study whether the obesity paradox might also prevail among RTR, we explored effect modification by BMI on the associations of UUE with graft failure and mortality. No effect modification was found by BMI on the association of UUE with mortality. In contrast, the association of UUE with graft failure was most pronounced in RTR with a BMI less than 25 kg/m^2. Hence, we speculate that at some point, the adverse effects of malnutrition counterbalance the supposed renoprotective effects of low protein intake in RTR.

It is plausible that high protein intake might be a marker of a healthy diet, low in saturated fat, low in sugar, or high in fibre. Since we did not collect information on diet, we cannot discern whether this was the case in our study. In addition, we did not have information on the type of protein ingested. We only collected data on sodium excretion, a marker for sodium intake, which was positively associated with protein intake. The associations of UUE with graft failure and mortality remained after adjustment for sodium intake. Although we adjusted for potential confounders, we cannot exclude the possibility of residual confounding. Worth mentioning is that we found UUE positively associated with delayed graft function, while it was inversely associated with ischemia time. This is somewhat unexpected because long ischemia time is an acknowledged risk factor for delayed graft function. We have no good explanation for this observation.

Some limitations of this study need to be addressed. First, a unique asset of this study was that we obtained data from regular patient care. As a consequence, we did not have data on inflammatory markers, body composition and energy intake which are of considerable interest. Second, UUE was used as a marker of protein intake. Commonly, protein intake is estimated with Maroni's equation, taking also body weight into account (34). However, Maroni's equation was validated for subjects with chronic renal disease only (34). Therefore, we estimated protein intake using a regression equation obtained from a different study of RTR. Another limitation is that we have no repeated measurements of UUE.

In most epidemiological studies like ours, a baseline classification for a parameter that may not be stable over time is used to study potential associations with outcome (35,36). It is generally acknowledged that this may adversely affect strength of associations with outcomes and even lead to false negation of otherwise truly existing associations (35,36). We, however, found a significant association despite these shortcomings of using tertiles of UUE. Finally, in observational studies, cause-effect relationships cannot be ascertained. The strength of the present study is that UUE was measured multiple times over a 6-month period. Using the median of multiple measurements reduces measurement errors. Moreover, we included a large number of stable RTR, and the follow-up period exceeded that of many other studies. Another strength of our study is that there was no loss to follow-up.

In conclusion, UUE, a marker for protein intake, was not associated with graft failure, but was inversely associated with graft failure in RTR with a BMI less than 25 kg/m^2 and in RTR with an eGFR of 45 mL per min per 1.73 m^2 or higher. Furthermore, RTR with UUE below 20.1 g per 24 hr had the highest mortality rates. These findings need to be confirmed in other prospective cohorts and in intervention studies. In the meantime, it may be better to avoid low protein intake in RTR and be heedful of a habitual low protein intake in RTR.

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Supplementary Table S1. Conversion equations for results of laboratory measurements performed on Merck Mega Analyzer or Roche Modular.

Measurement	Conversion equation
Urinary urea	$Y^a = (X^b - 9) / 0.996$
Total cholesterol	No difference
Serum albumin	$Y^a = (X^b + 6) / 1.132$
Serum creatinine	$Y^a = (X^b - 8) / 1.07$
Urinary protein	$Y^a = (X^b + 0.05) / 1.403$
Urinary sodium	$Y^a = X^b / 0.918$
Urinary creatinine	No difference

[a]Roche Modular (Roche Ltd., Mannheim, Germany)

[b]Merck Mega Analyzer (Merck, Darmstadt, Germany)

Conversion equations are presented in SI units.

Supplementary Table S2. Cox regression analyses for graft failure according to BMI-stratified tertiles of urinary urea excretion, in subjects with BMI <25 kg/m^2.

	BMI-stratified tertiles of urinary urea excretion[a]			
	I	II	III	
	Reference category	HR (95% CI)	HR (95% CI)	*P*-value[b]
No. of events	16	10	5	
Model 1	1.0	0.67 (0.30–1.47)	0.37 (0.14–1.01)	0.001
Model 2	1.0	0.57 (0.25–1.28)	0.31 (0.11–0.88)	<0.001
Model 3	1.0	0.60 (0.26–1.36)	0.29 (0.10–0.81)	<0.001
Model 4	1.0	0.64 (0.28–1.50)	0.27 (0.09–0.83)	<0.001

[a]Range of urinary urea excretion (g/24h) in tertiles: I, ≤18.4; II, 18.4–22.8; III, ≥ 22.8

[b]P for trend is shown

Model 1: Crude association

Model 2: Model 1 + age, sex

Model 3: Model 2 + donor age, and donor sex

Model 4: Model 3 + urinary sodium excretion and estimated GFR

Abbreviations: HR, hazard ratio; CI, confidence interval

Supplementary Table S3. Cox regression analyses for graft failure according to BMI-stratified tertiles of urinary urea excretion, in subjects with BMI ≥ 25 kg/m^2.

	BMI-stratified tertiles of urinary urea excretion[a]			
	I	II	III	
	Reference category	HR (95% CI)	HR (95% CI)	*P*-value[b]
No. of events	17	14	14	
Model 1	1.0	0.86 (0.42–1.74)	0.98 (0.48–1.98)	0.59
Model 2	1.0	0.80 (0.39–1.66)	0.95 (0.46–1.97)	0.58
Model 3	1.0	0.83 (0.40–1.71)	1.00 (0.48–2.09)	0.73
Model 4	1.0	1.10 (0.51–2.34)	1.31 (0.57–3.04)	0.78

[a]*Range of urinary urea excretion (g/24h) in tertiles: I, ≤ 21.1; II, 21.1–25.7; III, ≥ 25.8*

[b]*P for trend is shown*

Model 1: Crude association

Model 2: Model 1 + age, sex

Model 3: Model 2 + donor age, and donor sex

Model 4: Model 3 + urinary sodium excretion and estimated GFR

Abbreviations: HR, hazard ratio; CI, confidence interval

Supplementary Table S4. Cox regression analyses for graft failure according to eGFR-stratified tertiles of urinary urea excretion, in subjects with eGFR <45 ml/min/1.73m^2.

	eGFR-stratified tertiles of urinary urea excretion[a]				
	I	II		III	
	Reference category	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
No. of events	20	9		15	
Model 1	1.0	0.39 (0.18–0.86)	0.020	0.85 (0.43–1.66)	0.63
Model 2	1.0	0.45 (0.20–1.02)	0.055	1.02 (0.48–2.13)	0.97
Model 3	1.0	0.45 (0.20–1.01)	0.054	0.97 (0.46–2.04)	0.94
Model 4	1.0	0.41 (0.18–0.94)	0.035	0.95 (0.40–2.27)	0.91

[a]*Range of urinary urea excretion (g/24h) in tertiles: I, ≤20.1; II, 20.1–23.7; III, ≥ 23.7*

Model 1: Crude association

Model 2: Model 1 + age, sex

Model 3: Model 2 + donor age, and donor sex

Model 4: Model 3 + urinary sodium excretion and body mass index

Abbreviations: HR, hazard ratio; CI, confidence interval

Supplementary Table S5. Cox regression analyses for graft failure according to eGFR-stratified tertiles of urinary urea excretion, in subjects with eGFR ≥ 45 ml/min/1.73m^2.

	eGFR-stratified tertiles of urinary urea excretion[a]				
	I	II		III	
	Reference category	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
No. of events	16	10		8	
Model 1	1.0	0.61 (0.28–1.35)	0.23	0.60 (0.26–1.40)	0.24
Model 2	1.0	0.53 (0.24–1.19)	0.12	0.55 (0.23–1.32)	0.18
Model 3	1.0	0.47 (0.21–1.07)	0.071	0.55 (0.23–1.34)	0.19
Model 4	1.0	0.34 (0.15–0.79)	0.015	0.31 (0.11–0.86)	0.025

[b]*Range of urinary urea excretion (g/24h) in tertiles: I, ≤22.3; II, 22.3–24.1; III, ≥ 24.1*

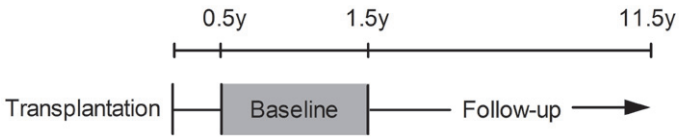
Model 1: Crude association

Model 2: Model 1 + age, sex

Model 3: Model 2 + donor age, and donor sex

Model 4: Model 3 + urinary sodium excretion and body mass index

Abbreviations: HR, hazard ratio; CI, confidence interval



Supplementary Figure S1. Study design. The median of laboratory measurements between 0.5 year (y) and 1.5 years after transplantation was used for analyses. From 1.5 years after transplantation, follow-up was recorded until the incidence of death, or after 10 years of follow-up.