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ORIGINAL ARTICLE

Urinary sulfate excretion and risk of late graft failure in renal transplant recipients – a prospective cohort study

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ABSTRACT

Hydrogen sulfide (H_2S), produced from metabolism of dietary sulfur-containing amino acids, is allegedly a renoprotective compound. Twenty-four-hour urinary sulfate excretion (USE) may reflect H_2S bioavailability. We aimed to investigate the association of USE with graft failure in a large prospective cohort of renal transplant recipients (RTR). We included 704 stable RTR, recruited at least 1 year after transplantation. We applied log-rank testing and Cox regression analyses to study association of USE, measured from baseline 24 h urine samples, with graft failure. Median age was 55 [45–63] years (57% male, eGFR was 45 ± 19 ml/min/1.73 m^2). Median USE was 17.1 [13.1–21.1] mmol/24 h. Over median follow-up of 5.3 [4.5–6.0] years, 84 RTR experienced graft failure. RTR in the lowest sex-specific tertile of USE experienced a higher rate of graft failure during follow-up than RTR in the middle and highest sex-specific tertiles (18%, 13%, and 5%, respectively, log-rank $P < 0.001$). In Cox regression analyses, USE was inversely associated with graft failure [HR per 10 mmol/24 h: 0.37 (0.24–0.55), $P < 0.001$]. The association remained independent of adjustment for potential confounders, including age, sex, eGFR, proteinuria, time between transplantation and baseline, BMI, smoking, and high sensitivity C-reactive protein [HR per 10 mmol/24 h: 0.51 (0.31–0.82), $P = 0.01$]. In conclusion, this study demonstrates a significant inverse association of USE with graft failure in RTR, suggesting high H_2S bioavailability as a novel, potentially modifiable factor for prevention of graft failure in RTR.

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Key words
chronic graft failure, hydrogen sulfide, protein intake, renal transplantation

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Introduction

Renal transplantation has been a lifesaving treatment for end-stage renal disease for over 60 years [1]. Improvements in prevention and treatment of short-term complications and overall greater quality of life have made it the preferred treatment over dialysis [2]. The long-term survival after renal transplantation, however, has disappointingly remained almost unchanged over the years [3,4], with half of renal transplant recipients (RTR) experiencing graft failure (defined as return to dialysis or retransplantation) or death within a decade after transplantation [5]. This late graft failure is not only a personal disaster [6], but also a significant burden for the healthcare system, because, nowadays, return to dialysis as a consequence of a failing graft is one of the most common reasons for initiation of dialysis [7,8]. This stresses the importance of identifying potentially modifiable risk factors for premature graft failure.

Hydrogen sulfide (H_2S), an endogenously produced gaseous compound from the conversion of the sulfuric amino acids cysteine and methionine, has been shown to have cytoprotective properties [9,10]. The cytoprotective properties of H_2S are based on its anti-inflammatory, antioxidant, and anti-apoptotic effects, which may at least in part be mediated by its ability to directly scavenge reactive oxygen species (ROS) and downregulate the ROS-producing enzymes [11]. For instance, the H_2S donor NaHS has been shown to suppress expression of the ROS-generating enzyme NADPH oxidase (NOX) and its essential subunit Rac-1. NaHS has also been shown to attenuate homocysteine-induced oxidative stress by inhibiting ROS production and NOX-4 protein expression [12]. Furthermore, NaHS has been shown to have a synergistic effect on the antioxidant properties of other agents such as apocynin, superoxide dismutase, and N-acetyl-cysteine [12]. In addition, H_2S exhibits anti-inflammatory effects. For example, treatment of murine cells exposed to pro-inflammatory LPS with the slow-release H_2S donor GYY4137 resulted in a concentration-dependent reduction of the of production of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 [13]. Furthermore, specific renoprotective properties of H_2S have been shown in mice models with hyperhomocysteinemia, where H_2S supplementation reduced both macrophage infiltration and collagen deposition in glomeruli [14].

Urinary sulfate excretion (USE) represents flux through the H_2S pathway, with sulfate being an end-metabolite of H_2S metabolism [15]. Consistent with cytoprotective properties on renal tissue, it has been found that high USE, reflecting high flux through this pathway, has been associated with lower rates of decline of renal function and lower risk of developing renal failure in patients with type 1 and 2 diabetes mellitus [16,17]. We hypothesized that a similar protective effect might be present in RTR and set out to investigate the association of USE with graft failure in RTR. Secondary aim was to study associations of USE with change of renal function over time.

Materials and methods

Study population

The cohort is part of a larger prospective cohort study (the Transplantlines Food and Nutrition Biobank and Cohort Study, Clinicaltrials.gov NCT02811835) of RTR in the northern part of the Netherlands. Adult RTR aged ≥18 years with a functioning graft for at least one year after transplantation and who visited the outpatient clinic of the University Medical Center Groningen, the Netherlands, between November 2008 and March 2011 were invited (n = 817). Exclusion criteria were history of drug or alcohol abuse, overt congestive heart failure, an (earlier) diagnosis of malignancy other than cured skin cancer, and insufficient understanding of the Dutch language. From these, 706 RTR agreed to participate and signed written informed consent. We excluded those without data on USE, leaving 704 RTR available for this study. Baseline was defined as the first visit for laboratory and physical measurements. All RTR were in a steady state at time of these measurements and provided written informed consent. The study protocol was approved by the Institutional Ethical Review Board (METc 2008/186), and the study has been conducted in accordance with the Declaration of Helsinki and Istanbul.

Laboratory measurements

We used routine laboratory methods for plasma and urinary laboratory measurements, as used in clinical practice and described as earlier [18,19]. Blood was drawn after an overnight fasting period of 8–12 h. Twenty-four-hour urine was collected for the urinary laboratory measurements.

We measured USE using an ion-exchange chromatography assay with conductivity detection (Metrohm, type 861, Herisau, Switzerland) which is described previously [18,20,21]. In short, urinary samples that had been

stored frozen at −80 °C until assessment were defrosted, centrifuged, and diluted 50 times with ultrapure water. After homogenization, 20 µl of the diluted sample was injected using a spark Triathlon autosampler (Spark Holland, Triathlon, Emmen, the Netherlands). Sulfate was separated from other urinary components with a Metrosep A supp 10 (100/4.0) column, using a 5 mmol/l sodium hydrogen carbonate/sodium carbonate buffer at a flow of 0.85 ml/min. Sulfate was detected using conductivity detection (Metrohm, type 861). Intra- and inter-assay coefficients of variation were 2.0% and 4.3%, respectively. Estimated GFR was calculated with serum creatinine and cystatin C concentrations using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula [22]. Proteinuria was defined as urinary excretion of ≥0.5 g/24 h. Protein intake at baseline was calculated using the Maroni equation and 24 h urinary urea excretion data [23,24].

Anthropomorphic and clinical measurements

Waist (cm) was measured between the 10th rib and the iliac crest on bare skin. Body weight was measured in kg, body height in meters without shoes. Body mass index (BMI) was calculated as weight/height^2. Body surface area was calculated in m^2 with the Du Bois and Du Bois formula [25]. Blood pressure was measured in fasting state in the morning in half-sitting position with a Dinamap® 1846 monitor (Critikon, Tampa, FL, USA) and recorded as the average of the last three of 15 successive measurements (with one-minute intervals between the measurements).

Smoking status was categorized as never, ex or current. Alcohol intake was measured by questionnaire and reported as one of three categories: 0–10 g/24 h, 10–30 g/24 h, and >30 g/24 h. Diabetes mellitus was diagnosed according to American Diabetes Association criteria: fasting plasma glucose ≥7.0 mmol/l and/or glycated hemoglobin (HbA1c) ≥6.5%, or use of antidiabetic medication [26].

Outcome measurements

The primary outcome during follow-up was death-censored graft failure which was defined as insufficient transplant function necessitating the return to dialysis or retransplantation. There were 124 cases (18% of study population) censored due to death prior to end of follow-up. The outcomes were assessed at the yearly visit to the outpatient clinic of the University Medical Center Groningen, the Netherlands. Secondary outcomes were the associations of USE with change of renal function parameters during follow-up, using 2 time points beyond baseline, with eGFR (CKD-EPI equation) and serum creatinine data available. End-points were recorded until September 30, 2015, after which the database was locked.

Statistical analysis

We used IBM SPSS statistics version 23 (2015; IBM Corp., Armonk, NY, USA), R statistics version 3.2.3 (2015, R Foundation for Statistical Computing, Vienna, Austria), and GRAPHPAD PRISM version 5.04 for Windows (2010; GraphPad Software, La Jolla, CA, USA). Univariable linear regression analyses were performed on the associations of USE with baseline characteristics. For the primary analyses, we used Kaplan–Meier and log-rank tests to test the differences in outcomes between sex-specific tertiles of USE and we used Cox proportional hazard analyses to study the associations between USE with graft failure. In order to estimate median follow-up, we also applied reverse Kaplan–Meier analyses, according to Schemper and Smith [27]. In these analyses, the indicators for the censor and the events of the Kaplan–Meier analyses are reversed. We used partially cumulative models to avoid a low number of events per variable [28]: Basic confounding variables were included cumulatively up to model 1, after which all the subsequent models included potential confounding variables in addition to model 1 only. The proportionality of hazards assumption was checked using the Schoenfeld residuals test and was not violated. Interactions of USE with age, sex, BMI, eGFR, alcohol intake, urinary protein excretion, and urea excretion were tested by introducing a product term of the previously mentioned variables and USE in the Cox regression model.

For secondary analyses of potential associations of USE with change of renal function, we used mixed linear model analyses with data of eGFR (CKD-EPI) and serum creatinine over three measuring moments. The associations were additionally adjusted for potential confounders as in the primary analyses. We used an unstructured covariance structure and assigned the intercept and slope of the models as random. We calculated predictive margins of eGFR and serum creatinine over time [29]. The predictive margins [+95% confidence interval (CI)] for eGFR and serum creatinine were stratified according to tertiles of USE and plotted to give a more easily interpretable presentation of the associations.

Data are presented as mean ± standard deviation (SD) for normally distributed variables, median

[interquartile range (IQR)] for non-normally distributed variables, and nominal data as number (percentages). For Cox proportional hazard analyses, hazard ratios (HR) and 95% CI are presented. For the linear regression associations, standardized coefficients (st. β) are presented. A *P*-value of ≤0.05 is regarded statistically significant.

Results

General basic characteristics at baseline are presented in Table 1 and transplant-related basic characteristics in Table 2. Median USE was 17.1 [IQR: 13.1–21.1] mmol/24 h. Men had higher USE than women (18.9 [14.8–22.6] vs. 15.0 [11.9–19.0] mmol/24 h). Median age was 55 [45–63] years, and 57% of the 704 RTR were male. In univariable linear regression, USE was positively associated with the male gender, larger body dimensions, diastolic blood pressure, alcohol intake, urea excretion, and protein intake (Table 1). Figure 1 shows the association of urinary sulfate excretion with protein intake. Also, it was positively associated with living kidney donation, prednisolone dosage, and eGFR (Table 2). USE was inversely associated with blood triglyceride, high sensitivity C-reactive protein (hs-CRP), leukocyte count, and N-terminal pro-brain natriuretic peptide levels (Table 1). It was also inversely associated with time between transplantation and baseline, cold ischemia time, repetitive transplantations, serum creatinine, and proteinuria (Table 1).

USE and graft failure

Median follow-up time after baseline was 5.4 (IQR: 4.9–6.1) years according to the reverse Kaplan–Meier method. Out of 704 RTR, 84 (12%) experienced graft failure during follow-up. The most common cause of graft failure was chronic rejection (73.8%), followed by recurrence of primary renal disease (9.5%), acute rejection (3,6%), graft infection (3,6%), other infection (3,6%), non-operable vascular problems (2.4%), and other nonspecified causes (3,6%). In Fig. 2, Kaplan–Meier analysis of the associations of sex-specified tertiles of USE with graft failure is presented. Higher sex-specific tertiles of USE is associated with less cumulative risk of graft failure (Log-Rank: $P < 0.001$).

Cox proportional hazard analyses of the association of USE with graft failure are presented in Table 3. USE is inversely associated with risk of graft failure [HR (95% CI) per 10 mmol/24 h: 0.37 (0.24–0.55), $P < 0.001$]. This association remained independent of adjustment for potential confounders, including age and baseline eGFR, as well as alcohol intake as an extragenous source of sulfate (Models 1–4). In addition, the association of USE with graft failure was independent of 24 h urinary urea excretion [Model 5: HR (95% CI) per 10 mmol/24 h: 0.34 (0.15–0.78), $P = 0.01$]. No significant interaction terms were found for the selected covariates (All $P > 0.05$).

USE and renal function decline

Higher USE was associated with a higher predicted eGFR and lower predicted serum creatinine at baseline (Tables S1 and S2). USE was also associated with less predicted increase of serum creatinine over time, but not with predicted decline of eGFR over time (Table S2). Figure 3 provides a visualization of the associations of USE with predicted eGFR and serum creatinine and their change over time, stratified over tertiles of USE: RTR in the highest tertile have significantly less predicted increase of serum creatinine compared to RTR in the lower tertiles.

Discussion

In this prospective cohort study with 704 stable RTR, we found that USE is inversely associated with graft failure. Unadjusted, there is a risk reduction of 63% for graft failure per 10 mmol/24 h increment of USE. The association of USE with graft failure remained independent of adjustment for potential confounders. To our knowledge, this is the first time that the associations of USE with graft failure have been studied in RTR. In secondary analyses, we studied the association of USE with eGFR and serum creatinine over time. There was a significant association between USE with less serum creatinine increase over follow-up time. The positive association of USE with eGFR was only significant at baseline. These results largely corroborate the findings of van den Born *et al.* [17] who found that USE was associated with higher eGFR and less risk of end-stage renal disease in a large cohort of patients with type 2 diabetes. Van den Berg *et al.* [18] have shown before in the same study cohort that higher excretion of urinary sulfur metabolites, in particular, USE, is associated with beneficial cardiovascular parameters and less all-cause mortality.

The biological pathway through which these findings are realized may be the H_2S pathway. Specific renoprotective benefits of H_2S may be protection against ischemia–reperfusion damage, involving among others downregulation of coagulation and stress response genes [30-32]. Snijder *et al.* [33] have shown that in hypertensive rats, administration of sodium thiosulfate reduces

Table 1. Basic characteristics.

	n = 704	St. β of univariable association with 24 h sulfate excretion	*P*
Sulfate excretion, mmol/24 h	17.10 [13.05–21.12]	n/a	n/a
Men	18.89 [14.76–22.60]	n/a	n/a
Women	15.03 [11.89–19.04]	n/a	n/a
Demographics			
Age of patient (years)	54.6 [44.9–62.9]	−0.03	0.46
Male gender, *n* (%)	400 (56.8%)	0.27	*<0.001*
Body composition			
Weight, kg	80.4 ± 16.6	0.29	*<0.001*
BMI, kg/m^2	26.0 [23.2–29.4]	0.15	*<0.001*
BSA, m^2	1.94 ± 0.22	0.34	*<0.001*
Blood pressure			
Systolic pressure, mmHg	136 ± 18	−0.03	0.49
Diastolic pressure, mmHg	83 ± 11	0.09	*0.02*
Use of antihypertensives, *n* (%)	621 (88.2)	−0.01	0.76
Number of antihypertensive drugs, *n* (%)			
0 antihypertensive drugs	82 (11.6)	Ref.	Ref.
1 antihypertensive drugs	194 (27.6)	0.02	0.77
≥2 antihypertensive drugs	428 (60.8)	0.02	0.77
Lipids			
Total cholesterol, mmol/l	5.13 ± 1.13	−0.04	0.26
HDL cholesterol, mmol/l	1.30 [1.10–1.60]	−0.02	0.53
LDL cholesterol, mmol/l	2.99 ± 0.93	0.004	0.92
Triglycerides, mmol/l	1.68 [1.25–2.29]	−0.10	*0.01*
Use of statins, *n* (%)	373 (53.0)	0.002	0.96
Diabetes (at baseline)			
Diabetes, *n* (%)	168 (23.9)	−0.05	0.23
Use of antidiabetic drugs, *n* (%)	109 (15.5)	−0.07	0.06
Glucose, mmol/l	5.3 [4.8–6.0]	−0.02	0.68
HbA1c, %	5.8 [5.5–6.2]	−0.02	0.70
Inflammation			
Hs-CRP, mg/l	1.60 [0.70–4.60]	−0.08	*0.04*
Blood leukocyte, $\times 10^9$/l	8.13 ± 2.62	−0.02	0.53
Cardiovascular disease history			
Myocardial infarction*, *n* (%)	35 (5)	−0.03	0.50
CABG and/or PCI, *n* (%)	55 (7.8)	−0.04	0.35
CVA or TIA, *n* (%)	41 (5.8)	−0.01	0.79
Heart failure			
NT-proBNP, ng/l	254 [109–623]	−0.10	*0.01*
Smoking status, *n* (%)†			
Never or ex	578 (82.1)	Ref.	Ref.
Current	83 (11.8)	−0.01	0.85
Alcohol intake, *n* (%)†			
0–10 g/day	472 (67.0)	Ref.	Ref.
10–30 g/day	139 (19.7)	0.13	*0.001*
>30 g/day	30 (4.3)	0.11	*0.01*
Urea excretion, mmol/24 h	388 ± 114	0.85	*<0.001*
Total protein intake, g/kg body weight/day	1.09 ± 0.27	0.56	*<0.001*

BMI, body mass index; BSA, body surface area; CABG, coronary artery bypass grafting; hs-CRP, high-sensitivity C-reactive protein; CVA, cardiovascular accident; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NT-proBNP, N-terminal pro-brain natriuretic peptide; PCI, percutaneous coronary intervention; TIA, transient ischemic attack.
Data are presented as mean ± standard deviation (SD) for normally distributed variables, median [interquartile range (IQR)] for variables with a skewed distribution, and nominal data as number (percentages).
Significant *P* values are displayed in italics.
*STEMI and/or NSTEMI.
†Percentages do not add up to 100% due to missing cases.

Table 2. Transplant-related characteristics.

	(*n* = 704)	St. β of univariable association with 24 h sulfate excretion	*P*
Time between transplantation and baseline, years	5.3 [1.9–12.0]	−0.13	*0.001*
(Pre)transplant history			
Pretransplant disease*, *n* (%)			
Primary glomerular disease	198 (28.1)	0.02	0.66
Glomerulonephritis	54 (7.7)	0.02	0.71
Tubular interstitial disease	84 (11.9)	−0.08	0.10
Polycystic renal disease	146 (20.7)	−0.02	0.77
Dysplasia and hypoplasia	28 (4.0)	−0.01	0.88
Renovascular disease	40 (5.7)	−0.01	0.81
Diabetes mellitus	36 (5.1)	−0.06	0.15
Other/unknown cause	118 (16.8)	Ref	
Dialysis time, months	41.7 ± 25.0	−0.05	0.56
Donor type, *n* (%)			
Living donor	240 (34.1)	0.09	*0.02*
Ischemia times			
Cold ischemia times (h)	15.3 [2.8–21.2]	−0.09	*0.02*
Warm ischemia times (min)	40 [33–50]	0.02	0.64
Initial immunosuppression after transplantation, *n* (%)†			
Corticosteroids	22 (3.1)	−0.05	0.26
Ciclosporin A	188 (26.7)	−0.09	0.15
Tacrolimus	3 (0.4)	<0.001	1.00
ATG	60 (8.5)	−0.02	0.65
OKT3 monoclonal AB‡	16 (2.3)	−0.05	0.22
Anti-IL2R monoclonal AB	348 (49.4)	0.04	0.60
Rituximab	2 (0.3)	−0.02	0.59
Other	27 (3.8)	Ref.	Ref.
Rejection after transplantation up to baseline, *n* (%)	188 (26.7)	−0.06	*0.12*
Number of transplantations, *n* (%)			
1	635 (90.2)	Ref.	Ref.
2 or more	69 (9.8)	−0.07	*0.05*
Immunosuppressive medication			
Prednisolone dosage, mg/24 h	10.0 [7.5–10.0]	0.10	*0.01*
CNI usage§, *n* (%)	404 (57.4)	−0.01	0.79
Proliferation inhibitor¶, *n* (%)	584 (83.0)	0.06	0.12
Renal allograft function			
Serum urea, mmol/l	9.6 [7.2–13.4]	−0.04	0.34
Serum creatinine, μmol/l	125 [100–161]	−0.11	*0.01*
eGFR, ml/min/1.73 m^2	45.0 ± 18.7	0.20	*<0.001*
Protein excretion, g/24 h	0.20 [0.02–0.37]	−0.09	0.02
Proteinuria (>0.5 g per 24 h), *n* (%)	158 (22.4)	−0.12	*0.002*

AB, antibody; ATG, antithymocyte globulin; CNI, calcineurin inhibitor; eGFR, estimated glomerular filtration rate; IL2r, interleukin-2 receptor.

eGFR was calculated according to the CKD-EPI creatinine and cystatin C formula. Data are presented as mean ± standard deviation (SD) for normally distributed variables, median [interquartile range (IQR)] for variables with a skewed distribution, and nominal data as number (percentages).

Significant *P* values are displayed in italics.

*Percentages do not add up to 100% because of rounding.

†Percentages do not add up to 100% because of missing cases.

‡Muromonab-CD3.

§e.g. tacrolimus.

¶e.g. mycophenolate mofetil.

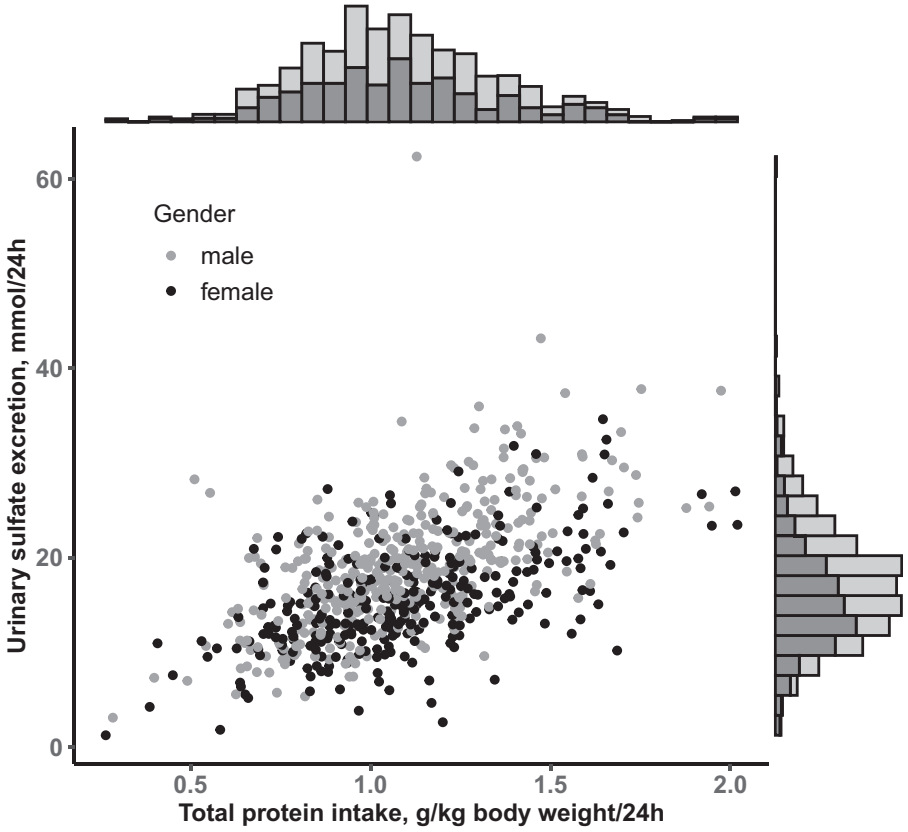
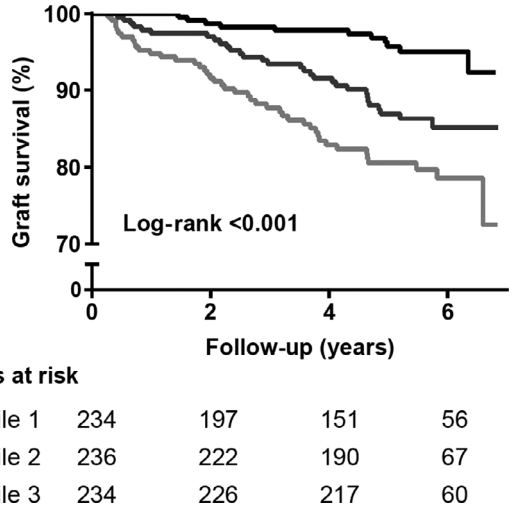


Figure 1 Scatterplot of total protein intake, calculated with the urea nitrogen excretion-derived Maroni formula, and urinary sulfate excretion.



Subjects at risk

Tertile 1	234	197	151	56
Tertile 2	236	222	190	67
Tertile 3	234	226	217	60

Figure 2 Kaplan–Meier analyses of the associations of sex-specific tertiles of urinary sulfate excretion with graft survival censored for death.

oxidative stress along with reduction of blood pressure, a risk factor of late graft failure [34], and cardiac fibrosis [33]. Sen *et al.* [14] have shown in hyperhomocysteinemic mice that supplementation of H_2S as sodium hydrogen sulfide in drinking water attenuated the observed adverse effects of hyperhomocysteinemia (e.g., blood pressure increase), reduction of inflammatory markers, and glomerular macrophage infiltration.

Dietary sources of sulfate are sulfur-containing amino acids via animal or plant protein or sulfate-rich foods(-components), such as beer and preservatives. We found that USE has a very high correlation with urinary urea excretion, which reflects total protein intake in stable RTR [24,35,36]. Additionally, USE is also strongly correlated to estimated total protein intake which can be calculated with the urea nitrogen excretion-derived Maroni formula (Fig. 1) [23]. A somewhat weaker correlation has been found for the association of USE with alcohol intake. Alcoholic beverages can be a source of exogenous sulfate intake. Earlier, alcohol intake has been found to be protective for all-cause mortality in RTR, but has no protective effect for graft failure [37]. Over the entire cohort, USE was independently associated with lower risk of graft failure. This association was independent of adjustment for alcohol intake. We conclude that the beneficial effect of USE on reducing the risk of premature graft failure is mainly dependent upon sulfur intake through dietary protein. Our current findings corroborate an earlier study of us where we found that low protein intake, as reflected by low 24 h urinary urea excretion, is associated with increased risk for premature graft failure in RTR [35,36]. The current guideline of the "Kidney Disease: Improving Global Outcomes" workgroup does not provide specific recommendations

Table 3. Cox proportional hazard analyses of USE (per 10 mmol 24 h) with graft failure.

Models	HR [95% CI]	*P*
Crude	0.37 [0.24–0.55]	<0.001
Model 1	0.51 [0.31–0.82]	0.01
Model 2	0.53 [0.33–0.85]	0.01
Model 3	0.50 [0.31–0.82]	0.01
Model 4	0.57 [0.35–0.93]	0.03
Model 5	0.34 [0.15–0.78]	0.01
Model 1	Crude + age, sex, baseline eGFR (creatinine and cystatin C), proteinuria, time from transplantation to baseline, BMI, smoking (never/ex or current), and hs-CRP	
Model 2	Model 1 + donor type (living versus postmortal), CIT, number of transplantations at baseline, and prednisolone dosage	
Model 3	Model 1 + diastolic blood pressure, triglycerides, and NT-proBNP	
Model 4	Model 1 + alcohol intake (0–10 g/day, 10–30 g/day, or >30 g/day)	
Model 5	Model 1 + urea excretion	

BMI, body mass index; CI, confidence interval; CIT, cold ischemia time; hs-CRP, high sensitivity C-reactive protein; eGFR, estimated glomerular filtration rate; HR, hazard ratio; NT-proBNP, N-terminal pro-brain natriuretic peptide; USE, urinary sulfate excretion.

Data are presented as hazard ratio [95% confidence interval]. A table with parameters of all covariables can be found in Table S3.

regarding protein intake for RTR [38], neither does the guideline of the "National Kidney Foundation Kidney Disease Outcomes Quality Initiative" (NKF KDOQI) [39,40]. Although it should be realized that the observational nature of our study does not allow for conclusions on cause–effect relationships, our findings suggest that high USE, especially from dietary protein sources high in sulfur-containing amino acids (methionine and cysteine), could be protective against development of graft failure. Methionine and cysteine are found in higher concentrations in animal-based protein compared to plant-based protein [41]. However, at this point, how much animal protein intake would be required to achieve a meaningful reduction in risk remains inconclusive. Also, potential risks of increased animal protein intake still need to be investigated first before recommending an increased animal-based protein intake for RTR. Future intervention studies could provide more insight into whether a diet high in methionine and cysteine is truly renoprotective.

Apart from the potential role of H_2S, another interesting potential explanation of the associations revealed in the present study is that a higher sulfate excretion represents higher sulfate bioavailability for sulfate-conjugation of endogenous and exogenous substances, thereby increasing their water solubility and facilitating renal excretion. Levy *et al.* [42] found that 20–30% of moderate doses of acetaminophen (1–2 g) is metabolized to its sulfur-conjugated metabolite and that the availability of sulfate was the capacity-limiting step. Kuchel *et al.* [43] have shown that small intravenously administered dosages of dopamine are predominantly sulfate conjugated with little to no increase in serum-free dopamine and blood pressure. Sulfate conjugation may therefore also function as a buffering mechanism for the variation in serum catecholamine concentrations and thereby blood pressure regulation [43,44].

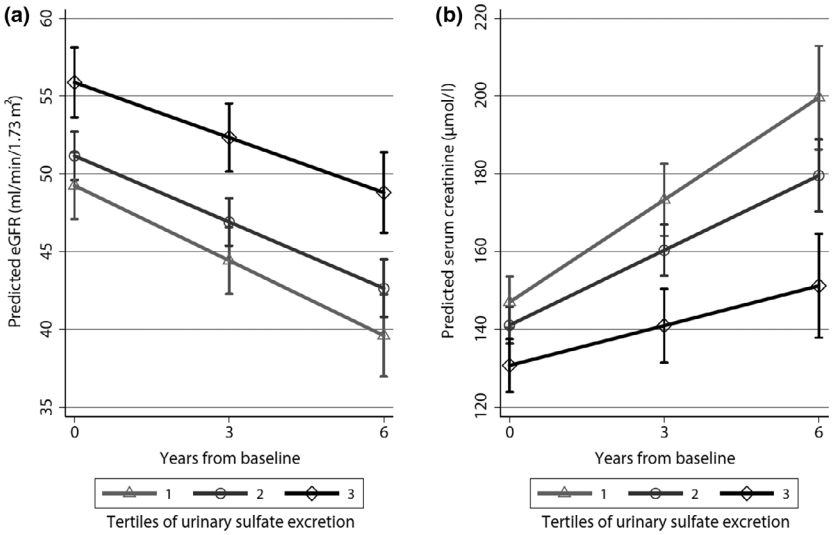


Figure 3 Predicted margins of renal function markers over follow-up time for tertiles of urinary sulfate excretion. (a) Predicted margins (+95% CI) of eGFR over time for sex-specific tertiles of urinary sulfate excretion. (b) Predicted margins (+95% CI) of serum creatinine over time for sex-specific tertiles of urinary sulfate excretion. The slopes of the tertiles of (a) do not significantly differ from each other ($P = 0.11$), while those of (b) are significantly different ($P < 0.001$).

Strengths of this study are its prospective study design, long follow-up, and large study population. The ability to adjust for renal function over time using linear mixed-effects modeling of eGFR and serum creatinine adds an important dimension to the analysis of the association of USE with graft failure. A limitation of our study is that we did not include RTR that experienced graft failure in the time between transplantation and baseline measurements. We found a positive association of USE with time between transplantation and baseline measurements. To compensate for the potential effect of healthy survivorship on USE and outcomes, we adjusted the association of USE with graft failure for the individual time between transplantation and baseline. Although the results remained materially unaffected, residual confounding caused by healthy survivor bias cannot be excluded.

Another limitation of this study is that we were not able to adjust for gasotransmitters other than H_2S. Nitric oxide metabolism, for example, may have cross talk with H_2S metabolism, although whether this is of mainly synergistic or antagonistic nature is still under debate and dependent of organ system and tissue concentrations [45]. Furthermore, we could not accurately investigate the association of specific type of protein intake. Animal protein sources are of higher quality and the found associations may be linked to higher intake of animal protein [46]. A general limitation of this study and all observational studies is that the found associations do not necessarily imply causality.

Conclusions

We have found that USE is strongly associated with reduced risk of graft failure. Potential explanations are the beneficial effects of an increased H_2S bioavailability through protein intake, or sulfate acting as a detoxifier or catecholamine buffering mechanism. These results indicate that sulfur intake may play an important role after renal transplantation and that an increase of sulfur-containing amino acids intake may have benefits for long-term graft survival. However, further research is warranted to determine the mechanism underlying this association and to investigate the effects of specific dietary sources of sulfate on long-term outcome in RTR.

Authorship

MYS: analyzed the data and drafted the manuscript. AP: participated in data collection and manuscript preparation. IM: participated in data collection; MVL: analyzed the data. HVG: revised the paper and participated in study design. DP: participated in statistical analyses. MRH-F: critically revised the article. EB: designed the data base, participated in subject care, and participated in data collection. AP: supervised in data collection and critically revised the article. GN: critically revised the article. SJLB: initiated the study, supervised data collection, participated in subject care, and edited the manuscript.

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Conflicts of interest

The authors declare no conflict of interest.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Linear Mixed Models for the association of urinary sulfate excretion (per 10 mmol/24 h) and eGFR (CKD-EPI creatinine-based formula) over follow-up time.

Table S2. Linear Mixed Models for the association of urinary sulfate excretion (per 10 mmol/24 h) and serum creatinine over follow-up time.

Table S3. Cox proportional hazard analyses of the association of USE with graft failure, including parameters of all covariables.

REFERENCES

1. Doyle AM, Lechler RI, Turka LA. Organ transplantation: halfway through the first century. *J Am Soc Nephrol* 2004; **15**: 2965.
2. Pesavento TE. Kidney transplantation in the context of renal replacement therapy. *Clin J Am Soc Nephrol* 2009; **4**: 2035.
3. Meier-Kriesche HU, Schold JD, Srinivas TR, Kaplan B. Lack of improvement in renal allograft survival despite a marked decrease in acute rejection rates over the most recent era. *Am J Transplant* 2004; **4**: 378.
4. Hariharan S, Johnson CP, Bresnahan BA, Taranto SE, McIntosh MJ, Stablein D. Improved graft survival after renal transplantation in the United States, 1988 to 1996. *N Engl J Med* 2000; **342**: 605.
5. Matas AJ, Gillingham KJ, Humar A, *et al.* 2202 kidney transplant recipients with 10

years of graft function: what happens next? *Am J Transplant* 2008; **8**: 2410.
6. Howell M, Wong G, Rose J, Tong A, Craig JC, Howard K. Patient preferences for outcomes after kidney transplantation: a best-worst scaling survey. *Transplantation* 2017; **101**: 2765.
7. States Renal Data System. *2015 USRDS annual data report: Epidemiology of Kidney Disease in the United States*. Bethesda, MD: National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, 2015.
8. Perl J. Kidney transplant failure: failing kidneys, failing care? *Clin J Am Soc Nephrol* 2014; **9**: 1153.
9. Snijder PM, van den Berg E, Whiteman M, Bakker SJL, Leuvenink HGD, van Goor H. Emerging role of gasotransmitters in renal transplantation. *Am J Transplant* 2013; **13**: 3067.
10. Koning AM, Frenay ARS, Leuvenink HG, van Goor H. Hydrogen sulfide in renal physiology, disease and transplantation–the smell of renal protection. *Nitric Oxide Biol Chem* 2015; **46**: 37.
11. Aminzadeh MA, Vaziri ND. Downregulation of the renal and hepatic hydrogen sulfide (H_2S)-producing enzymes and capacity in chronic kidney disease. *Nephrol Dial Transpl* 2012; **27**: 498.
12. Tyagi N, Moshal KS, Sen U, *et al.* H_2S protects against methionine-induced oxidative stress in brain endothelial cells. *Antioxid Redox Signal* 2009; **11**: 25.
13. Whiteman M, Li L, Rose P, Tan CH, Parkinson DB, Moore PK. The effect of hydrogen sulfide donors on lipopolysaccharide-induced formation of inflammatory mediators in macrophages. *Antioxid Redox Signal* 2010; **12**: 1147.
14. Sen U, Munjal C, Qipshidze N, Abe O, Gargoum R, Tyagi SC. Original report: laboratory investigation hydrogen sulfide regulates homocysteine-mediated glomerulosclerosis. *Am J Nephrol* 2010; **31**: 442.
15. Szabó C. Hydrogen sulphide and its therapeutic potential. *Nat Rev Drug Discov* 2007; **6**: 917.
16. Andrésdóttir G, Bakker SJL, Hansen HP, Parving H-H, Rossing P. Urinary sulphate excretion and progression of diabetic nephropathy in Type 1 diabetes. *Diabet Med* 2013; **30**: 563.
17. van den Born JC, Frenay ARS, Bakker SJL, *et al*. High urinary sulfate concentration is associated with reduced risk of renal disease progression in type 2 diabetes. *Nitric Oxide Biol Chem* 2016; **55–56**: 18.
18. van den Berg E, Pasch A, Westendorp WH, *et al*. Urinary sulfur metabolites associate with a favorable cardiovascular risk profile and survival benefit in renal transplant recipients. *J Am Soc Nephrol* 2014; **25**: 1303.
19. van den Berg E, Engberink MF, Brink EJ, *et al*. Dietary acid load and metabolic acidosis in renal transplant recipients. *Clin J Am Soc Nephrol* 2012; **7**: 1811.
20. Farese S, Stauffer E, Kalicki R, *et al*. Sodium thiosulfate pharmacokinetics in hemodialysis patients and healthy volunteers. *Clin J Am Soc Nephrol* 2011; **6**: 1447.
21. Newton GL, Dorian R, Fahey RC. Analysis of biological thiols: derivatization with monobromobimane and separation by reverse-phase high-performance liquid chromatography. *Anal Biochem* 1981; **114**: 383.
22. Inker LA, Schmid CH, Tighiouart H, *et al*. Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med* 2012; **367**: 20.
23. Maroni BJ, Steinman TI, Mitch WE. A method for estimating nitrogen intake of patients with chronic renal failure. *Kidney Int* 1985; **27**: 58.
24. van den Berg E, Engberink MF, Brink EJ, *et al*. Dietary protein, blood pressure and renal function in renal transplant recipients. *Br J Nutr* 2013; **109**: 1463.
25. Du Bois D, Du Bois EF. A formula to estimate the approximate surface area if height and weight be known. *Nutrition* 1916; **5**: 303; discussion 312-3.
26. Gaudreau S, Michaud C. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2008; **31**: S55.
27. Schemper M, Smith TL. A note on quantifying follow-up in studies of failure time. *Control Clin Trials* 1996; **17**: 343.
28. Concato J, Peduzzi P, Holford TR, Feinstein AR. Importance of events per independent variable in proportional hazards analysis. I. Background, goals, and general strategy. *J Clin Epidemiol* 1995; **48**: 1495.
29. Graubard BI, Korn EL. Predictive margins with survey data. *Biometrics* 1999; **55**: 652.
30. Lobb I, Davison M, Carter D, *et al*. Hydrogen sulfide treatment mitigates renal allograft ischemia-reperfusion injury during cold storage and improves early transplant kidney function and survival following allogeneic renal transplantation. *J Urol* 2015; **194**: 1806.
31. Zhu JXG, Kalbfleisch M, Yang YX, *et al*. Detrimental effects of prolonged warm renal ischaemia-reperfusion injury are abrogated by supplemental hydrogen sulphide: an analysis using real-time intravital microscopy and polymerase chain reaction. *BJU Int* 2012; **110**: E1218.
32. Han SJ, Kim JI, Park J-W, Park KM. Hydrogen sulfide accelerates the recovery of kidney tubules after renal ischemia/reperfusion injury. *Nephrol Dial Transplant* 2015; **30**: 1497.
33. Snijder PM, Frenay ARS, De Boer RA, *et al*. Exogenous administration of thiosulfate, a donor of hydrogen sulfide, attenuates angiotensin II-induced hypertensive heart disease in rats. *Br J Pharmacol* 2015; **172**: 1494.
34. Opelz G, Wujciak T, Ritz E. Association of chronic kidney graft failure with recipient blood pressure. *Kidney Int* 1998; **53**: 217.
35. Deetman PE, Said MY, Kromhout D, *et al*. Urinary urea excretion and long-term outcome after renal transplantation. *Transplantation* 2015; **99**: 1009.
36. Said MY, Deetman PE, de Vries APJ, *et al*. Causal path analyses of the association of protein intake with risk of mortality and graft failure in renal transplant recipients. *Clin Transplant* 2015; **29**: 447.
37. Zelle DM, Agarwal PK, Ramirez JLP, *et al*. Alcohol consumption, new onset of diabetes after transplantation, and all-cause mortality in renal transplant recipients. *Transplantation* 2011; **92**: 203.
38. Kidney Disease Improving Global Outcomes (KDIGO) Transplant Work Group. Special issue: KDIGO clinical practice guideline for the care of kidney transplant recipients. *Am J Transplant* 2009; **9**: S1.
39. Kopple JD. National kidney foundation K/DOQI clinical practice guidelines for nutrition in chronic renal failure. *Am J Kidney Dis* 2001; **37**: S66.
40. National Kidney Foundation. Clinical Practice Guideline for Nutrition in Chronic Kidney Disease: 2019 Update. https://www.kidney.org/professionals/kdoqi-guidelines-commentary-nutrition. Accessed on 11-01-2020.
41. Berrazaga I, Micard V, Gueugneau M, Walrand S. The role of the anabolic properties of plant- versus animal-based protein sources in supporting muscle mass maintenance: a critical review. *Nutrients* 2019; **11**: 1825.
42. Levy G, Yamada H. Drug biotransformation interactions in man. 3. Acetaminophen and salicylamide. *J Pharm Sci* 1971; **60**: 215.
43. Kuchel O, Buu NT, Serri O. Sulfoconjugation of catecholamines, nutrition, and hypertension. *Hypertension* 1982; **4**: III93.
44. Kuchel O, Buu NT, Racz K, De Léan A, Serri O, Kyncl J. Role of sulfate conjugation of catecholamines in blood pressure regulation. *Fed Proc* 1986; **45**: 2254.
45. Lo Faro ML, Fox B, Whatmore JL, Winyard PG, Whiteman M. Hydrogen sulfide and nitric oxide interactions in inflammation. *Nitric Oxide Biol Chem* 2014; **41**: 38.
46. Elango R, Levesque C, Ball RO, Pencharz PB. Available versus digestible amino acids – new stable isotope methods. *Br J Nutr* 2012; **108** (Suppl): S306.