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The influence of renal transplantation on retained microbial–human co-metabolites

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ABSTRACT

Background. Colonic microbial metabolism contributes substantially to uraemic retention solutes accumulating in chronic kidney disease (CKD) and various microbial–human co-metabolites relate to adverse outcomes. The influence of renal transplantation on these solutes is largely unexplored.

Methods. We prospectively followed 51 renal transplant recipients at the time of transplantation, Day 7 and Months 3 and 12 post-transplantation. Serum levels of *p*-cresyl sulphate (PCS), *p*-cresyl glucuronide (PCG), indoxyl sulphate (IS), trimethylamine *N*-oxide (TMAO) and phenylacetylglutamine (PAG) were determined with liquid chromatography–tandem mass spectrometry. At each time point, transplant recipients were compared with CKD control patients matched for age, gender, diabetes mellitus and renal function. Determinants of serum levels were also compared between an unrelated cohort of 65 transplant recipients at Month 3 post-transplantation and CKD patients with 24-h urinary collection.

Results. Serum levels of the tested microbial–human co-metabolites significantly decreased following renal transplantation ($P < 0.001$). At each time point post-transplantation, serum levels of PCS, PCG, PAG and, to a lesser extent, IS, but not TMAO, were significantly lower in transplant recipients when compared with CKD control patients. Further analysis demonstrated significantly lower 24-h urinary excretion of these solutes in transplant recipients ($P < 0.001$). Also, renal clearances of PCG, IS, TMAO and PAG were significantly lower in transplant recipients without differences in estimated glomerular filtration rate.

Conclusions. Colonic microbiota-derived uraemic retention solutes substantially decrease following renal transplantation. The 24-h urinary excretion of these microbial–human co-metabolites is lower when compared with CKD patients, suggesting an independent influence of transplantation on intestinal uptake, a composite of colonic microbial metabolism and intestinal absorption. Renal solute handling may differ between transplant recipients and CKD patients.

Keywords: microbiota, renal transplantation

INTRODUCTION

It is well established that renal transplantation is superior to dialysis with respect to patient survival and quality of life [1, 2]. Although this is probably partly due to better clearance of so-called uraemic retention solutes, little data corroborate this claim. In an ongoing quest for solutes determining the syndrome of uraemia, there has been increasing interest in colonic microbial metabolism as an important contributor to these solutes [3, 4]. Both *p*-cresyl sulphate (PCS) and indoxyl sulphate (IS) have repeatedly been associated with mortality and cardiovascular disease in patients with chronic kidney disease (CKD), also supported by experimental studies [5–13]. Besides sulphate conjugation, these solutes are subjected to glucuronidation with *p*-cresyl glucuronide (PCG), another *p*-cresol derivative present in patients with renal dysfunction, possibly relating to worse survival [14, 15]. Recent data also point to a relationship between two other microbial–human

co-metabolites, i.e. trimethylamine *N*-oxide (TMAO) and phenylacetylglutamine (PAG), and adverse outcomes in patients with CKD [16–18].

Although it may be expected that serum levels of these solutes decrease following renal transplantation due to regaining of renal function, this has been largely unexplored. Furthermore, whether renal transplantation affects phase 2 metabolism (i.e. sulphation versus glucuronidation) of these microbial–human co-metabolites is also unknown. Finally, renal transplantation may have an impact on the colonic microbial composition itself [19, 20], although the influence on microbial metabolism and generation of these solutes remains unclear.

Therefore, in this study, we explored the natural history of selected colonic microbiota-derived uraemic retention solutes after renal transplantation. In addition, we aimed to investigate whether renal transplantation affects these microbial–human co-metabolites beyond renal function regain.

MATERIALS AND METHODS

Study population

We performed a prospective observational study in 51 renal allograft recipients transplanted at the University Hospitals Leuven (part of an ongoing trial, clinicaltrials.gov NCT01331668) to describe the natural post-transplant history of serum levels of selected colonic microbiota-derived uraemic retention solutes. Serum samples were collected at the time of transplantation and at Day 7 and Months 3 and 12 post-transplantation. At each time point, these 51 renal transplant recipients were compared with a control group of 51 CKD patients, matched for age, gender, presence of diabetes mellitus, dialysis modality and dialysis duration at the time of transplantation or renal function [serum creatinine, estimated glomerular filtration rate (eGFR) and measured creatinine clearance] at all other time points. Control patients were followed at the haemodialysis unit and the nephrology outpatient clinic of the University Hospitals Leuven and had been recruited in the frame of an ongoing epidemiological trial (clinicaltrials.gov NCT00441623). We also performed an additional analysis in an unrelated cohort of 65 stable renal transplant recipients at Month 3 post-transplantation in order to gain more insights into factors determining post-transplant serum levels of these microbial metabolites. The majority of renal transplant recipients (89.7%) received a standard immunosuppressive regimen consisting of tacrolimus, mycophenolate mofetil and steroids; all patients were given antimicrobial prophylaxis with cefazolin (perioperative), trimethoprim sulphamethoxazole (3 months post-transplantation) and valganciclovir (3 months post-transplantation, depending on donor–receptor cytomegalovirus serostatus). The study was performed according to the Declaration of Helsinki and approved by the ethics committee of the University Hospitals Leuven. The clinical and research activities being reported are consistent with the principles of the Declaration of Istanbul as outlined in the Declaration of Istanbul on Organ Trafficking and Transplant Tourism. Informed consent was obtained from all subjects.

Biochemical measurements

At each time point, blood was taken by venous puncture for the measurement of serum creatinine (mg/dL), albumin (g/L), total PCS (μM), total PCG (μM), total IS (μM), total TMAO (μM) and total PAG (μM). Serum creatinine and albumin were measured using standard laboratory techniques. The eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation [21]. Serum levels of PCS, PCG, IS, TMAO and PAG were quantified using a dedicated ultraperformance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS) method (Supplementary data). In the second cohort of 65 renal transplant recipients, we also sampled 24-h urinary collections, allowing us to determine renal clearance and 24-h urinary excretion for the selected microbial–human co-metabolites. Assuming steady-state conditions and negligible non-renal clearance, 24-h urinary excretion can be considered an estimate of the daily intestinal uptake of these solutes. The combined 24-h urinary excretion of PCS and PCG is representative of the daily intestinal uptake of their precursor *p*-cresol.

Statistical analysis

Data are expressed as mean (SD) for normally distributed variables or median [interquartile range (IQR)] for non-normally distributed variables. Correlations and differences between serum levels of microbial–human co-metabolites at different time points post-transplantation were tested with Spearman's rank correlation coefficient and the Wilcoxon signed-rank test, respectively. Differences in demographic and biochemical variables between renal transplant recipients and CKD control patients were determined using the Student's *t*-test, Wilcoxon rank-sum test or χ^2 test as appropriate. Linear regression analysis was performed to evaluate the contribution of eGFR and 24-h urinary excretion to serum solute levels at Month 3 post-transplantation. Correlations between eGFR and renal clearances of the tested microbial–human co-metabolites at Month 3 post-transplantation were investigated with Spearman's rank correlation coefficient.

RESULTS

Natural history of serum levels of microbial–human co-metabolites post-transplantation

We prospectively followed 51 renal transplant recipients (Supplementary data, Table S1) with the measurement of serum levels of PCS, PCG, IS, TMAO and PAG at the time of transplantation, Day 7 and Months 3 and 12 post-transplantation. Glomerular disease (37.3%) and congenital renal disease (including autosomal dominant polycystic disease; 29.4%) were the most frequent underlying causes of end-stage renal disease. Along with regaining renal function, demonstrated by a decline in serum creatinine, serum levels of PCS, PCG, IS, TMAO and PAG significantly decreased following renal transplantation ($P < 0.001$ for all solutes at each time point versus time of transplantation; Figure 1). In addition, the proportion of serum PCS to PCG changed after transplantation, with relatively higher serum levels of PCS at each time point post-transplantation

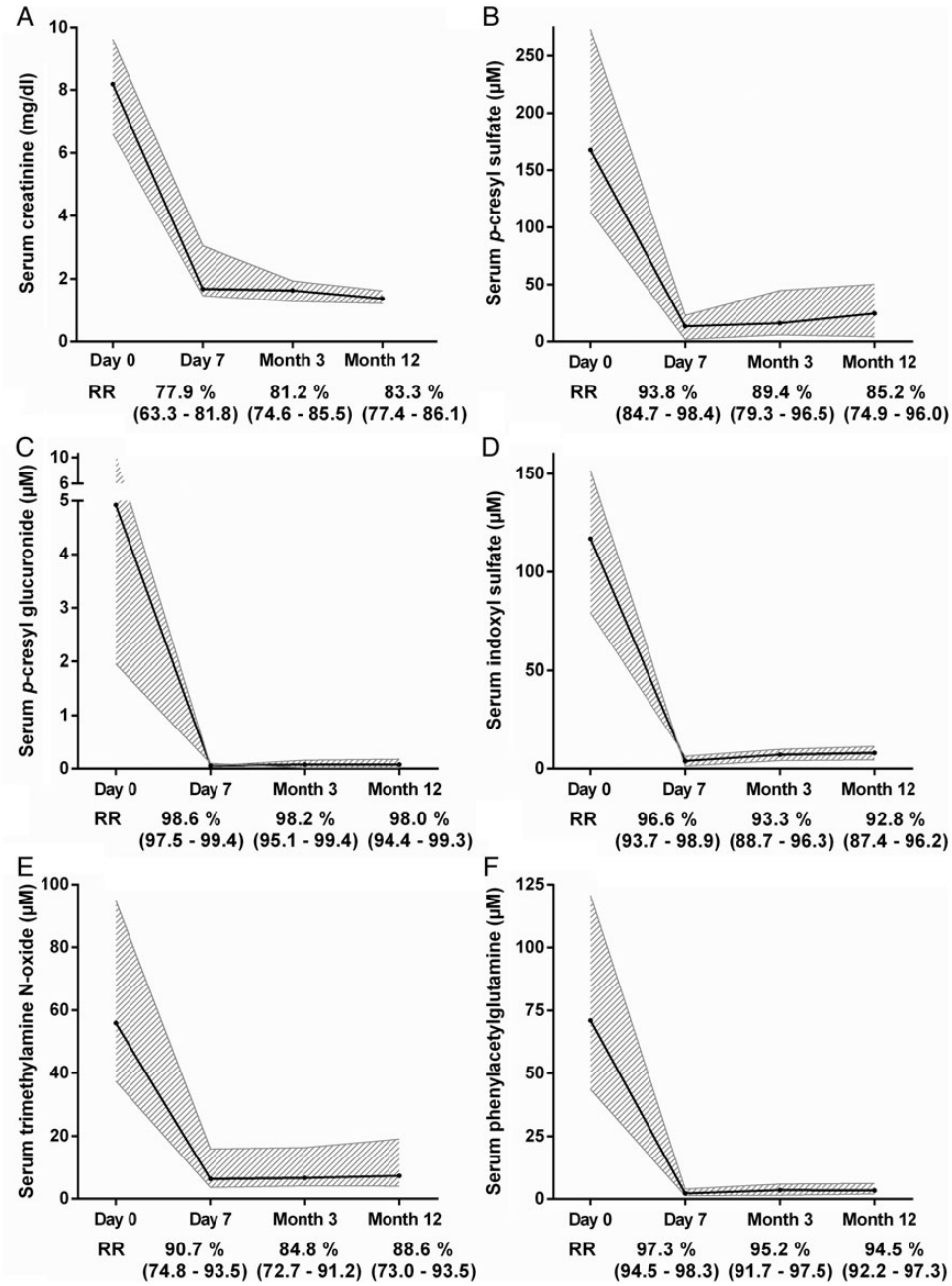

FIGURE 1: Natural history of serum levels of microbial–human co-metabolites after renal transplantation. Evolution of serum (**A**) creatinine, (**B**) *p*-cresyl sulphate, (**C**) *p*-cresyl glucuronide, (**D**) indoxyl sulphate, (**E**) trimethylamine *N*-oxide and (**F**) phenylacetylglutamine at the time of transplantation, Day 7 and Months 3 and 12 post-transplantation. Median, black line; IQR, grey area; RR, reduction ratio.

[median 35.3 (IQR 19.9–64.9) at the time of transplantation versus 179.5 (IQR 76.1–271.8) at Day 7, 187.2 (IQR 110.4–289.8) at Month 3 and 192.2 (IQR 99.7–371.6) at Month 12; $P < 0.001$ at each time point versus time of transplantation]. When compared with serum creatinine, reduction ratios for all solutes were nominally higher at each time point post-transplantation. We observed a sharp initial decrease in serum levels of all microbial–human co-metabolites between the time of transplantation and Day 7 post-transplantation, after which there was a small, albeit significant increase in serum levels of PCS, PCG, IS and PAG, but not of TMAO between Day 7 and Month 3 ($P = 0.01$ for PCS, $P = 0.03$ for PCG, $P < 0.001$ for IS, $P = 0.78$ for TMAO and $P = 0.002$ for PAG). There was no significant change in serum solute levels between Months 3 and 12 post-transplantation ($P = 0.23$ for PCS, $P = 0.36$ for PCG, $P = 0.52$ for IS, $P = 0.82$ for TMAO and $P = 0.36$ for PAG). We noted a significant correlation between serum PCS at the time of transplantation and all time points post-transplantation ($\rho = 0.34$, $P = 0.01$ at Day 7; $\rho = 0.51$, $P < 0.001$ at Month 3; $\rho = 0.38$, $P = 0.007$ at Month 12). Serum PCG at the time of transplantation was also significantly correlated with serum PCG at Month 3 ($\rho = 0.32$, $P = 0.02$) and Month 12 ($\rho = 0.29$, $P = 0.04$), but not with serum PCG at Day 7 post-transplantation ($\rho = 0.20$, $P = 0.16$). In contrast, there was no

correlation between pre- and post-transplant serum levels of IS ($\rho = -0.01$, P = 0.95 at Day 7; $\rho = 0.13$, P = 0.36 at Month 3; $\rho = 0.10$, P = 0.46 at Month 12). Serum TMAO at the time of transplantation was significantly correlated with serum TMAO at Month 12 ($\rho = 0.46$, P < 0.001) but not with serum TMAO at Day 7 ($\rho = 0.21$, P = 0.13) and Month 3 post-transplantation ($\rho = 0.11$, P = 0.43). A significant correlation was also observed between pre- and post-transplant serum levels of PAG ($\rho = 0.33$, P = 0.01 at Day 7; $\rho = 0.36$, P = 0.009 at Month 3; $\rho = 0.36$, P = 0.01 at Month 12).

Renal transplant recipients versus CKD control patients

At each time point post-transplantation, the cohort of 51 renal transplant recipients was compared with 51 CKD control patients, matched for age, gender, presence of diabetes mellitus and eGFR. Besides higher serum levels of C-reactive protein (CRP) in renal transplant recipients at Day 7 post-transplantation, we observed no differences in inflammatory status at all other time points. To exclude potential differences in post-transplant serum levels of PCS, PCG, IS, TMAO and PAG solely due to dissimilarities already present prior to transplantation, we also matched this cohort at the time of transplantation with a control group of haemodialysis and peritoneal dialysis patients (Table 1). These comparisons allowed differentiating between a natural history of the serum levels post-transplantation and an artificial reverse natural history in CKD. Following transplantation, serum levels of PCS, PCG, IS and PAG were significantly lower in renal transplant recipients than in CKD patients at Day 7 (P < 0.001 for all solutes), Month 3 (P < 0.001 for PCS and PCG, P = 0.04 for IS and P = 0.005 for PAG) and Month 12 (P < 0.001 for PCS, P < 0.001 for PCG, P = 0.04 for IS and P = 0.03 for PAG; Figure 2). There were no significant differences in the proportion of serum PCS to PCG between renal transplant recipients and CKD control patients (P = 0.31 at Day 7, P = 0.27 at Month 3 and P = 0.81 at Month 12). For serum TMAO, we only noted significantly lower levels in renal transplant recipients at Day 7 (P = 0.007), but not at Month 3 (P = 0.50) and Month 12 post-transplantation (P = 0.87).

Determinants of serum levels of microbial–human co-metabolites in renal transplant recipients versus CKD control patients

To further explore the observed differences in post-transplant serum levels of microbial–human co-metabolites, we studied a second cohort of 65 stable renal transplant patients at Month 3 post-transplantation for which 24-h urinary collections were available. This cohort was matched with a CKD control group with respect to age, gender, presence of diabetes mellitus and eGFR (Table 2). There were neither between-group differences in serum levels of CRP. We observed no differences in 24-h urinary excretion of urea as an estimate of daily protein intake. Again, serum levels of PCS, PCG and PAG were significantly lower in renal transplant recipients than in CKD patients (P < 0.001 for both PCS and PCG and P = 0.05 for PAG). Serum IS and TMAO were not significantly different between both groups (P = 0.11 for IS and P = 0.14 for TMAO). As all these solutes are largely dependent on intestinal uptake and renal clearance, 24-h urinary excretion, which in steady-state conditions approximates daily intestinal uptake, and renal clearance were calculated in both groups. For the tested microbial–human co-metabolites, 24-h urinary excretion was significantly lower in renal transplant recipients (P < 0.001 for all solutes). While there were no between-group differences in renal clearance of PCS (P = 0.22), we noted a significantly lower renal clearance of PCG (P < 0.001), IS (P = 0.04), TMAO (P < 0.001) and PAG (P = 0.02) in renal transplant recipients. When performing regression analysis with renal function, measured by eGFR, and 24-h urinary excretion, 24-h urinary excretion was a significant determinant of serum levels of all solutes, while the contribution of eGFR was less pronounced or even absent in renal transplant recipients when compared with CKD control patients (Table 3). Also, correlations between eGFR and renal clearances of microbial–human co-metabolites were nominally higher in the CKD control group than in renal transplant recipients (Table 4).

DISCUSSION

In this study, we explored the influence of renal transplantation on serum levels of colonic microbiota-derived uraemic retention solutes, focusing on PCS, PCG, IS, TMAO and PAG. The key findings are as follows: (i) serum levels of microbial–human co-metabolites substantially decrease following renal transplantation; (ii) serum levels of PCS, PCG, PAG, and, to a lesser extent, IS are lower in renal transplant recipients when compared with CKD patients; (iii) renal transplantation, including potential effects of immunosuppressive and antimicrobial drug therapy, diminishes 24-h urinary excretion as an indirect estimate of intestinal solute exposure and (iv) renal handling of the tested microbial–human co-metabolites is markedly different between renal transplant recipients and CKD patients.

There has been mounting evidence that colonic microbial metabolism contributes substantially to uraemic retention solutes accumulating in patients with renal dysfunction [3, 4]. Various microbial–human co-metabolites, including PCS, PCG, IS, TMAO and PAG, have also been related to CKD-related adverse outcomes [5–9, 15–18], although the influence of renal transplantation on their serum levels has been largely unexplored. Therefore, we prospectively followed a cohort of 51 renal transplant recipients at the time of transplantation and at Day 7 and Months 3 and 12 post-transplantation. Along with serum creatinine decline, we observed a significant decrease in serum PCS, PCG, IS, TMAO and PAG following renal transplantation. Reduction ratios for the tested solutes were higher than for serum creatinine, pointing to adequate post-transplant tubular secretion and/or diminished intestinal uptake of these microbial–human co-metabolites. As it is unknown whether renal transplantation affects serum levels of microbial–human co-metabolites independent of renal function regain, we also compared these patients with CKD control patients, matched for age, gender, presence of diabetes mellitus, nutritional parameters and renal function. At each time point post-transplantation, serum PCS, PCG, PAG and, to a lesser extent, IS, but not TMAO, remained significantly lower in renal transplant recipients than in CKD counterparts. Although there was a change in the proportion of serum PCS to PCG post-transplantation, with relatively higher serum levels of

Table 1. Comparison between renal transplant recipients and CKD control patients ($N = 51$), matched at the time of transplantation, Day 7 and Months 3 and 12 post-transplantation

Variable	Transplant recipients	CKD control patients	P-value
Time of transplantation			
Age (years)	52 (11)	54 (16)	0.35
Gender: male/female (%)	33/18 (64.7/35.3)	33/18 (64.7/35.3)	1
Dialysis type: HD/PD (%)	34/17 (66.7/33.3)	34/17 (66.7/33.3)	1
Dialysis duration (months)	39 (21)	39 (34)	0.98
Diabetes: yes/no (%)	6/45 (11.8/88.2)	6/45 (11.8/88.2)	1
Body mass index (kg/m^2)	25.3 (4.5)	24.4 (4.3)	0.28
Albumin (g/L)	43.1 (4.3)	41.8 (2.4)	0.06
C-reactive protein (mg/L)	3.3 (1.0–6.8)	3.6 (1.4–7.4)	0.42
Serum PCS (μM)	167.4 (113.4–273.4)	167.5 (112.6–254.1)	0.71
Serum PCG (μM)	4.92 (1.96–10.44)	7.74 (1.95–11.27)	0.35
Serum IS (μM)	116.9 (79.2–151.6)	133.3 (74.0–166.3)	0.90
Serum TMAO (μM)	55.9 (37.4–94.9)	84.5 (54.4–140.9)	0.02
Serum PAG (μM)	71.0 (43.6–120.8)	88.0 (46.4–145.3)	0.43
Day 7 post-transplantation			
Age (years)	52 (11)	51 (13)	0.63
Gender: male/female (%)	33/18 (64.7/35.3)	33/18 (64.7/35.3)	1
Creatinine (mg/dL)	1.68 (1.46–3.05)	2.11 (1.35–3.49)	0.49
eGFR (mL/min/1.73 m^2)	37 (23–51)	32 (17–56)	0.61
Creatinine clearance (mL/min)	38 (31–54)	33 (20–63)	0.45
Diabetes: yes/no (%)	6/45 (11.8/88.2)	6/45 (11.8/88.2)	1
Body mass index (kg/m^2)	25.7 (4.4)	25.4 (5.0)	0.78
Albumin (g/L)	35.1 (3.2)	44.5 (4.5)	<0.001
C-reactive protein (mg/L)	6.4 (2.4–16.6)	2.0 (1.0–6.0)	<0.001
Urea (mg/dL)	84 (57–127)	80 (41–128)	0.29
Serum PCS (μM)	13.4 (1.9–22.8)	66.3 (35.3–146.1)	<0.001
Serum PCG (μM)	0.06 (0.02–0.10)	0.29 (0.12–1.05)	<0.001
Serum IS (μM)	4.0 (1.4–6.4)	17.6 (8.1–34.4)	<0.001
Serum TMAO (μM)	6.3 (3.6–15.9)	14.5 (4.6–33.6)	0.007
Serum PAG (μM)	2.2 (1.3–4.0)	7.5 (3.3–23.2)	<0.001
Month 3 post-transplantation			
Age (years)	52 (11)	56 (16)	0.12
Gender: male/female (%)	33/18 (64.7/35.3)	33/18 (64.7/35.3)	1
Creatinine (mg/dL)	1.63 (1.28–1.93)	1.76 (1.17–2.21)	0.44
eGFR (mL/min/1.73 m^2)	45 (35–58)	39 (28–66)	0.26
Creatinine clearance (mL/min)	52 (40–65)	48 (33–63)	0.21
Diabetes: yes/no (%)	6/45 (11.8/88.2)	6/45 (11.8/88.2)	1
Body mass index (kg/m^2)	25.1 (4.2)	25.2 (4.7)	0.87
Albumin (g/L)	44.0 (3.2)	43.0 (4.7)	0.20
C-reactive protein (mg/L)	1.0 (1.0–2.2)	2.0 (1.0–4.0)	0.47
Urea (mg/dL)	60 (47–82)	65 (45–94)	0.59
Serum PCS (μM)	16.0 (5.9–44.9)	59.7 (27.0–89.7)	<0.001
Serum PCG (μM)	0.08 (0.02–0.16)	0.23 (0.10–0.45)	<0.001
Serum IS (μM)	7.2 (4.2–9.9)	11.0 (4.6–16.5)	0.04
Serum TMAO (μM)	6.6 (4.1–16.3)	10.6 (4.9–15.3)	0.50
Serum PAG (μM)	3.5 (1.5–5.9)	5.6 (2.7–12.0)	0.005
Month 12 post-transplantation			
Age (years)	53 (11)	54 (16)	0.65
Gender: male/female (%)	33/18 (64.7/35.3)	33/18 (64.7/35.3)	1
Creatinine (mg/dL)	1.37 (1.21–1.62)	1.40 (1.03–1.83)	0.85
eGFR (mL/min/1.73 m^2)	53 (41–62)	57 (35–76)	0.64
Creatinine clearance (mL/min)	66 (50–84)	61 (48–81)	0.30
Diabetes: yes/no (%)	6/45 (11.8/88.2)	6/45 (11.8/88.2)	1
Body mass index (kg/m^2)	25.7 (4.7)	26.0 (4.9)	0.80
Albumin (g/L)	44.5 (3.7)	44.5 (4.0)	0.93
C-reactive protein (mg/L)	1.6 (1.0–6.1)	2.0 (1.0–4.0)	0.84
Urea (mg/dL)	58 (50–71)	52 (35–83)	0.19
Serum PCS (μM)	24.5 (4.3–50.1)	56.0 (24.0–85.9)	<0.001
Serum PCG (μM)	0.08 (0.02–0.18)	0.24 (0.07–0.50)	<0.001
Serum IS (μM)	8.1 (4.6–11.4)	11.1 (5.7–15.4)	0.04
Serum TMAO (μM)	7.3 (4.0–19.0)	8.0 (4.5–14.6)	0.87
Serum PAG (μM)	3.4 (2.0–6.2)	5.8 (2.4–11.5)	0.03

Data are expressed as mean (SD) or median (IQR) as appropriate. Differences were tested using Student's *t*-test, Wilcoxon rank-sum test or χ^2 test as appropriate.
HD, haemodialysis; PD, peritoneal dialysis; eGFR, estimated glomerular filtration rate; PCS, *p*-cresyl sulphate; PCG, *p*-cresyl glucuronide; IS, indoxyl sulphate; TMAO, trimethylamine *N*-oxide; PAG, phenylacetylglutamine.

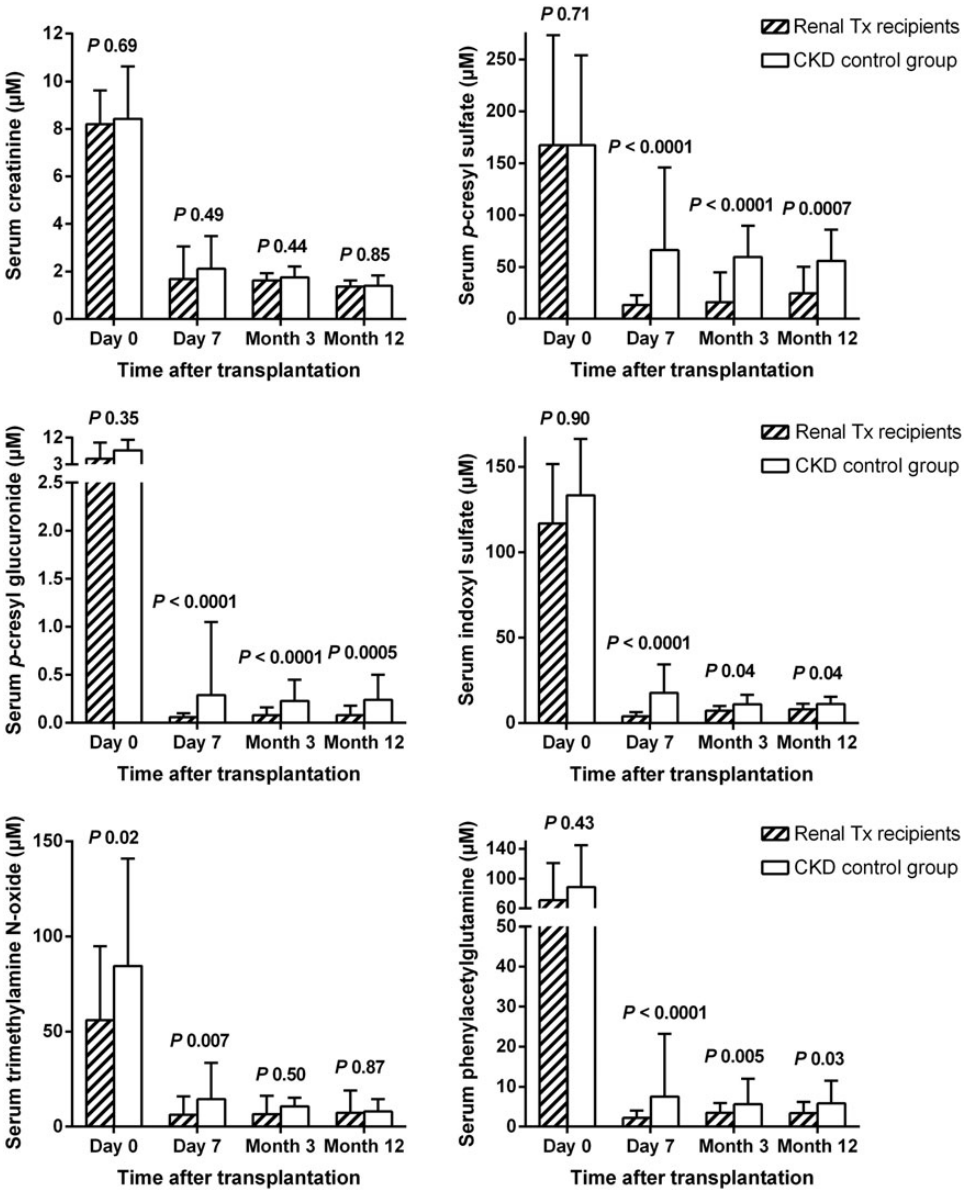

FIGURE 2: Serum levels of microbial–human co-metabolites in renal transplant recipients versus CKD control patients. Comparison of serum (**A**) creatinine, (**B**) *p*-cresyl sulphate, (**C**) *p*-cresyl glucuronide, (**D**) indoxyl sulphate, (**E**) trimethylamine *N*-oxide and (**F**) phenylacetylglutamine [median (IQR)] between renal transplant recipients and CKD control patients matched at each time point (time of transplantation, Day 7 and Months 3 and 12 post-transplantation).

PCS post-transplantation than at the time of transplantation, there were no differences in the proportion of serum PCS to PCG between renal transplant recipients and CKD control patients. From this, it can be inferred that the degree of renal function decline/regain, but not renal transplantation *per se*, influences phase 2 metabolism of *p*-cresol. Whether there is also no impact of renal transplantation on phase 2 metabolism of the other tested solutes needs further investigation.

Next, we studied 24-h urinary excretion of these microbial–human co-metabolites, which can be considered an indirect estimate of the daily intestinal uptake, assuming steady-state conditions and negligible non-renal clearance. As 24-h urinary collections were not available in the first cohort, we studied a second cohort of 65 stable renal transplant recipients at Month 3 post-transplantation with availability of 24-h urinary collections. The 24-h urinary excretion of the tested solutes was significantly lower in renal transplant recipients when compared with CKD control patients, indicative of a diminished total intestinal uptake of microbial–human co-metabolites following renal transplantation. Total intestinal uptake depends on intestinal generation, i.e. colonic microbial metabolism, and subsequent intestinal absorption. Although it has been suggested that the colonic microbial composition changes following renal transplantation [19, 20], less is known about microbial function. These findings may be indicative of a post-transplant shift in colonic microbial metabolism, which is also beyond renal function regain and diminishes intestinal generation of these solutes. This may not be surprising since renal transplant recipients, when compared with CKD counterparts, are subjected to transplant-specific factors that may influence

Table 2. Comparison between renal transplant recipients at Month 3 post-transplantation and CKD control patients ($N = 65$)

Variable	Transplant recipients	CKD control patients	P-value
Age (years)	53 (14)	54 (15)	0.72
Gender: male/female (%)	40/25 (61.5/38.5)	40/25 (61.5/38.5)	1
Creatinine (mg/dL)	1.49 (1.21–1.78)	1.44 (1.13–1.77)	0.70
eGFR (mL/min/1.73 m^2)	49 (40–63)	51 (39–66)	0.76
Creatinine clearance (mL/min)	52 (41–60)	53 (41–71)	0.28
24-h urinary excretion of creatinine (g)	1.05 (0.9–1.3)	1.10 (0.9–1.3)	0.40
Diabetes: yes/no (%)	8/57 (12.3/87.7)	8/57 (12.3/87.7)	1
Body mass index (kg/m^2)	24.3 (5.8)	25.4 (4.6)	0.26
Albumin (g/L)	43.5 (3.9)	44.4 (4.7)	0.26
C-reactive protein (mg/L)	1.3 (1.0–4.2)	1.0 (1.0–4.0)	0.38
Urea (mg/dL)	58 (46–70)	49 (39–67)	0.09
24-h urinary excretion of urea (g)	20.8 (15.3–26.8)	18.6 (15.5–23.2)	0.45
Serum PCS (μM)	8.8 (2.2–30.0)	35.7 (15.8–64.6)	<0.001
24-h urinary excretion of PCS (μmol)	116.3 (23.5–303.4)	559.5 (302.6–810.0)	<0.001
Clearance of PCS (mL/min)	8.8 (5.3–12.8)	9.7 (6.1–16.5)	0.22
Serum PCG (μM)	0.04 (0.02–0.13)	0.18 (0.05–0.31)	<0.001
24-h urinary excretion of PCG (μmol)	4.61 (1.52–20.66)	37.63 (15.18–76.87)	<0.001
Clearance of PCG (mL/min)	66.7 (34.5–134.1)	150.9 (98.6–266.9)	<0.001
24-h urinary excretion of *p*-cresol	120.8 (25.0–313.1)	596.0 (331.1–921.5)	<0.001
Serum IS (μM)	5.9 (3.7–10.7)	7.5 (3.9–13.4)	0.15
24-h urinary excretion of IS (μmol)	172.4 (108.3–242.7)	289.6 (178.2–416.3)	<0.001
Clearance of IS (mL/min)	21.4 (13.7–29.4)	26.7 (17.7–40.6)	0.04
Serum TMAO (μM)	8.3 (4.3–21.7)	6.7 (4.3–12.2)	0.14
24-h urinary excretion of TMAO (μmol)	206.8 (65.6–627.8)	532.5 (249.7–844.7)	0.008
Clearance of TMAO (mL/min)	24.2 (4.1–41.9)	49.0 (33.6–77.3)	<0.001
Serum PAG (μM)	2.8 (1.4–5.6)	4.3 (2.2–7.5)	0.05
24-h urinary excretion of PAG (μmol)	457.0 (309.3–880.9)	1256.6 (639.9–1539.5)	<0.001
Clearance of PAG (mL/min)	131.4 (100.2–199.5)	178.8 (105.7–261.7)	0.02

Data are expressed as mean (SD) or median (IQR) as appropriate. Differences were tested using Student's *t*-test, Wilcoxon rank-sum test or χ^2 test as appropriate. The 24-h urinary excretion of *p*-cresol equals the combined 24-h urinary excretion of PCS and PCG.
eGFR, estimated glomerular filtration rate; PCS, *p*-cresyl sulphate; PCG, *p*-cresyl glucuronide; IS, indoxyl sulphate; TMAO, trimethylamine *N*-oxide; PAG, phenylacetylglutamine.

microbial metabolism. For example, all renal transplant patients are prescribed antimicrobial prophylaxis and take immunosuppressive drug therapy. There has been mounting evidence that antimicrobial therapy is associated with a shift in colonic microbial composition and, therefore, may also affect post-transplant microbial metabolism [22]. Although recovery of the original microbiota can be observed after treatment discontinuation, this is often incomplete, thus not fully excluding a long-lasting effect on serum solute levels, even until 12 months post-transplantation, while, at our institution, antimicrobial prophylaxis is discontinued after 3 months. In addition, immunosuppressive drug therapy may influence colonic microbial metabolism. Since our cohort was treated with an almost uniform immunosuppressive drug regimen (i.e. combination of tacrolimus, mycophenolate mofetil and steroids), it was impossible to extract the potential individual effects of these immunosuppressive drugs. Furthermore, whether the effect of immunosuppressive drug therapy on microbial metabolism may either be direct or mediated through the interaction between microbial metabolism and the host immune system needs further investigation. Besides intestinal generation, total intestinal uptake also depends on intestinal absorption. Although mechanisms underlying intestinal absorption of these solutes has not been studied to date, they may theoretically include active intestinal transport and thus be potentially subjected to interaction with immunosuppressive drugs [23]. Furthermore, whether colonic mucosal changes, as are commonly observed with the use of mycophenolate mofetil, also influence intestinal absorption of microbial–human co-metabolites remains to be elucidated [24].

Although there were no between-group differences in eGFR and creatinine clearance, both glomerular renal function markers, we noted significantly lower renal clearances of most solutes in renal transplant recipients, even partly offsetting the effect of diminished intestinal uptake, especially for serum TMAO. There was also a clearly better correlation between eGFR and clearances of the selected microbial–human co-metabolites in CKD control patients when compared with renal transplant recipients. Thus, while eGFR is a suitable marker to estimate renal clearance of these solutes in patients with CKD [25], this may be different in renal transplant recipients. Various immunosuppressive drugs interact with renal tubular transporters that may also be involved in renal excretion of these microbial–human co-metabolites, thus potentially influencing renal solute clearance [26–29]. It could also be questioned whether these microbial–human co-metabolites influence renal excretion of immunosuppressive drugs and/or their metabolites, thereby affecting their systemic exposure, effectiveness and toxicity, a hypothesis that requires further investigation.

There are limitations to our study. First, to explore the impact of renal transplantation on colonic microbiota-derived uraemic retention solutes, we focused on PCS, PCG, IS, TMAO and PAG as representatives of this group of solutes. It can therefore not be excluded that renal transplantation will differentially

Table 3. Determinants of serum levels of microbial metabolites in renal transplant recipients at Month 3 post-transplantation and CKD control patients

Group	Variable	β-value	P-value
p-Cresyl sulphate			
Transplant recipients	eGFR (mL/min/1.73 m²) (Ln)	−0.84	0.05
	24-h urinary excretion (μmol) (Ln)	0.96	<0.001
		Model R^2	0.76
CKD control patients	eGFR (mL/min/1.73 m²) (Ln)	−1.32	<0.001
	24-h urinary excretion (μmol) (Ln)	0.91	<0.001
		Model R^2	0.84
p-Cresyl glucuronide			
Transplant recipients	eGFR (mL/min/1.73 m²) (Ln)	−0.09	0.82
	24-h urinary excretion (μmol) (Ln)	0.29	<0.001
		Model R^2	0.34
CKD control patients	eGFR (mL/min/1.73 m²) (Ln)	−1.19	0.001
	24-h urinary excretion of (μmol) (Ln)	0.42	<0.001
		Model R^2	0.49
Indoxyl sulphate			
Transplant recipients	eGFR (mL/min/1.73 m²) (Ln)	−0.60	0.01
	24-h urinary excretion of (μmol) (Ln)	0.92	<0.001
		Model R^2	0.70
CKD control patients	eGFR (mL/min/1.73 m²) (Ln)	−1.13	<0.001
	24-h urinary excretion of (μmol) (Ln)	0.76	<0.001
		Model R^2	0.69
Trimethylamine *N*-oxide			
Transplant recipients	eGFR (mL/min/1.73 m²) (Ln)	−0.12	0.81
	24-h urinary excretion of (μmol) (Ln)	0.15	0.04
		Model R^2	0.08
CKD control patients	eGFR (mL/min/1.73 m²) (Ln)	−0.85	0.001
	24-h urinary excretion of (μmol) (Ln)	0.25	<0.001
		Model R^2	0.38
Phenylacetylglutamine			
Transplant recipients	eGFR (mL/min/1.73 m²) (Ln)	−0.71	0.004
	24-h urinary excretion of (μmol) (Ln)	0.90	<0.001
		Model R^2	0.71
CKD control patients	eGFR (mL/min/1.73 m²) (Ln)	−1.43	<0.001
	24-h urinary excretion of (μmol) (Ln)	0.84	<0.001
		Model R^2	0.71

eGFR, estimated glomerular filtration rate.

Table 4. Spearman's rank correlation between eGFR and renal clearances of microbial metabolites in renal transplant recipients at Month 3 post-transplantation and CKD control patients

Variable	Transplant recipients		CKD control patients	
	ρ-value	P-value	ρ-value	P-value
PCS	0.39	0.003	0.68	<0.001
PCG	0.05	0.70	0.41	<0.001
IS	0.35	0.008	0.71	<0.001
TMAO	−0.04	0.78	0.51	<0.001
PAG	0.36	0.006	0.67	<0.001

eGFR, estimated glomerular filtration rate; PCS, *p*-cresyl sulphate; PCG, *p*-cresyl glucuronide; IS, indoxyl sulphate; TMAO, trimethylamine *N*-oxide; PAG, phenylacetylglutamine.

affect other microbial–human co-metabolites. Second, we estimated total intestinal uptake of the selected microbial–human co-metabolites by measuring 24-h urinary excretion, thereby assuming steady-state conditions and negligible non-renal clearance, which cannot be fully excluded. This approach also did not allow differentiation between intestinal generation and subsequent absorption. Third, as formal dietary assessment was not performed, we cannot exclude dietary differences between renal transplant recipients and CKD control patients. There were, however, no significant differences in 24-h urinary excretion of urea as an estimate of daily protein intake. Fourth, our study population mainly consisted of patients of Caucasian descent. Care must be taken to extrapolate our data to other patient populations. Finally, the number of renal transplant recipients in the study was relatively limited, prohibiting statements about potential relations between post-transplant solute levels and long-term allograft or patient outcome. However, a recent study, presented in abstract form, did not demonstrate a relationship between post-transplant serum levels of IS and adverse allograft or patient outcome [30].

In conclusion, colonic microbiota-derived uraemic retention solutes substantially decrease following renal transplantation. Intestinal uptake of these microbial–human co-metabolites, indirectly measured by 24-h urinary excretion, is also significantly lower when compared with CKD counterparts, suggesting an independent influence of renal transplantation on colonic microbial metabolism and/or intestinal absorption. In addition, renal solute handling is markedly different in renal transplant recipients. Whether these microbial–human co-metabolites are associated with adverse allograft or patient outcome necessitates further investigation.

SUPPLEMENTARY DATA

Supplementary data are available online at http://ndt.oxfordjournals.org.

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CONFLICT OF INTEREST STATEMENT

The results presented in this paper have not been published previously in whole or part, except in abstract form.

(See related article by Vanholder *et al.* Intestinal metabolites, chronic kidney disease and renal transplantation: Enigma Variations? *Nephrol Dial Transplant* 2016; 31: 1547–1551)

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