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Effect of a sustained difference in hemodialytic clearance on the plasma levels of p-cresol sulfate and indoxyl sulfate

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ORIGINAL ARTICLE

ABSTRACT

Background. The protein-bound solutes p-cresol sulfate (PCS) and indoxyl sulfate (IS) accumulate to high plasma levels in renal failure and have been associated with adverse events. The clearance of these bound solutes can be altered independently of the urea clearance by changing the dialysate flow and dialyzer size. This study tested whether a sustained difference in clearance would change the plasma levels of PCS and IS.

Methods. Fourteen patients on thrice-weekly nocturnal hemodialysis completed a crossover study of two periods designed to achieve widely different bound solute clearances. We compared the changes in pre-dialysis plasma PCS and IS levels from baseline over the course of the two periods.

Results. The high-clearance period provided much higher PCS and IS clearances than the low-clearance period (PCS: 23 ± 4 mL/min versus 12 ± 3 mL/min, $P < 0.001$; IS: 30 ± 5 mL/min versus 17 ± 4 mL/min, $P < 0.001$). Despite the large difference in clearance, the high-clearance period did not have a different effect on PCS levels than the low-clearance period [from baseline, high: +11% (−5, +37) versus low: −8% (−18, +32), (median, 25th, 75th percentile), $P = 0.50$]. In contrast, the high-clearance period significantly lowered IS levels compared with the low-clearance period [from baseline, high: −4% (−17, +1) versus low: +22% (+14, +31), $P < 0.001$]. The amount of PCS removed in the dialysate was significantly greater at the end of the high-clearance period [269 (206, 312) versus 199 (111, 232) mg per treatment, $P < 0.001$], while the amount of IS removed was not different [140 (87, 196) versus 116 (89, 170) mg per treatment, $P = 0.15$].

Conclusions. These findings suggest that an increase in PCS generation prevents plasma levels from falling when the dialytic clearance is increased. Suppression of solute generation may be required to reduce plasma PCS levels in dialysis patients.

Keywords: clearance, p-cresol sulfate, uremia

INTRODUCTION

Some solutes that accumulate in the plasma of hemodialysis patients bind to plasma proteins [1]. Such solutes are poorly cleared by hemodialysis because only the free, unbound portion diffuses across the dialyzer membrane. Several means of increasing their dialytic clearances have been investigated [2]. The two most extensively studied bound solutes, p-cresol sulfate (PCS) and indoxyl sulfate (IS), have been associated with mortality and cardiovascular events [3–7]. We previously showed that the dialytic clearance of bound solutes can be increased independent of the urea clearance by increasing the dialysate flow and dialyzer size [8]. In patients receiving conventional thrice-weekly treatment, use of two dialysis machines was required to achieve a large increase in dialysate flow [9]. In patients receiving longer-duration nocturnal hemodialysis, however, lower dialysate flows are routinely used so that a large proportional increase may be achieved without addition of a second machine [10]. We previously demonstrated that during single nocturnal dialysis treatments, bound solute clearances could be manipulated over a wide range while maintaining similar urea clearances [11]. This study tested whether sustained differences in bound solute clearance maintained over successive treatments would alter the plasma levels of PCS and IS.

MATERIALS AND METHODS

Patients were recruited from the in-center nocturnal hemodialysis program of Satellite Healthcare. Patients were excluded

if they were on antibiotics within 4 weeks, were vegetarian, had history of colon surgery, did not attend dialysis treatments regularly or had residual urea clearance >2 mL/min. The study was approved by the Institutional Review Board and was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all subjects.

Fourteen of 20 recruited patients completed two experimental periods in a crossover design. Six patients were excluded after recruitment: three had initial levels below the assay range for both PCS and IS, one patient was started on antibiotics, one patient transferred to another dialysis unit and one patient reduced his treatment time.

Each experimental period consisted of seven dialysis treatments, which were designed to achieve widely different protein-bound solute clearances but similar urea clearances, as summarized in Table 1. During Period A, dialysis was performed with blood flow 350 mL/min, dialysate flow 300 mL/min and an F160NR dialyzer, and during Period B, dialysis was performed with blood flow 270 mL/min, dialysate flow 800 mL/min and an F250NR dialyzer. Six patients started with Period A, and eight patients started with Period B. The experimental periods were separated by a washout period ranging from 42 to 91 days, during which the patients remained on their routine nocturnal hemodialysis prescriptions as summarized in Table 2. The dialysis time and ultrafiltration rate were the same during the experimental periods. The patients' diet and medications were not regulated.

Each period began and ended with a midweek treatment. Plasma samples were obtained pre-treatment at the first treatment of each period. At the last treatment of each period, plasma was obtained pre-treatment and post-treatment, and spent dialysate was collected during the entire treatment in large drums. Patients thus received six experimental treatments between the pre-treatment plasma measurements. PCS and IS were measured using liquid chromatography tandem mass spectrometry (LC/MS/MS) with stable isotopic dilution as previously described [12]. Plasma samples were deproteinized with 1:4 vol:vol methanol and diluted 50 and 10 times for the pre- and post-treatment samples, respectively, prior to analysis. Plasma ultrafiltrate was obtained using Nanosep 30K and diluted 5 and 2.5 times for the pre- and post-treatment samples, respectively. Dialysate fluid was diluted 2.5 times. Urea was measured using a commercial kit (Infinity™, Thermo Scientific). Plasma albumin and phosphate were measured by the clinical laboratory.

Table 1. Experimental dialysis prescriptions

	A	B
Dialysate flow (mL/min)	300	800
Dialyzer	F160NR	F250NR
Blood flow (mL/min)	350	270

Treatment duration and ultrafiltration remained the same for both periods.

Table 2. Baseline characteristics

Age (years)	57 ± 10
Diabetes mellitus (*n*)	10
Gender (M/F) (*n*)	13 / 1
Dialysis vintage (years)	3.9 ± 2.4
Nocturnal dialysis vintage (years)	2.0 ± 1.5
Blood flow (mL/min)	304 ± 13
Dialysate flow (mL/min)	477 ± 26
Dialyzer (F160/F180/F200) (*n*)	2/6/6
Dialysis duration (6/7.25/8 h) (*n*)	1/1/12
$spKt/V_{urea}$	2.6 ± 0.7
Hemoglobin (g/dL)	11.4 ± 1.8
Albumin (g/dL)	3.9 ± 0.3
Phosphate (mg/dL)	4.7 ± 1.3

Results are mean ± standard deviation unless otherwise indicated. All dialyzers were single use (non-reuse), and no patients had residual urea clearance >2 mL/min.

The effect of the experimental prescriptions on solutes levels was analyzed using repeated measures ANOVA. Patient characteristics, clearances, baseline solute levels, pre-dialysis percent binding and amount of solute removed in dialysate between the two periods were compared using the paired *t*-test. Data for free levels of IS from one subject were omitted from the final analysis as they were below the assay range. A P-value of <0.025 was considered significant to correct for the comparison of two solutes. Type II error and power to detect the expected change was calculated using a one-sample mean test with alpha 0.05, study sample size of 14 and standard deviation of the observed change as representative of the between-person variability. Statistical analysis was done using SPSS 23 and STATA 13.

Mathematical modeling was performed to predict the number of treatments required for plasma PCS levels to approach a new equilibrium and the effect on plasma levels of experimental periods including seven treatments, as depicted in Supplementary data, Figure S1 and in Figure 2. Modeling was initially performed assuming that solute generation remained constant, that there was no non-dialytic clearance and that the solutes were distributed in a single compartment the volume of which was estimated by dividing the amount of solute removed by the difference between the pre- and post-treatment plasma levels. Because we did not measure clearances while the patients were on their routine treatments, we estimated the baseline clearance by averaging the clearances measured at the end of the two experimental periods. Using different values for the estimated baseline clearance had very little effect on the modeled differences in plasma levels at the end of the two periods, as illustrated in Supplementary data, Figure S2. The estimated one-compartment volumes of distribution for PCS and IS ranged from 13 to 16 L. Employing different volumes in this range in a one-compartment model did not significantly affect the predicted differences in plasma levels. Eloot *et al.* [13] recently modeled the dialytic removal of PCS and IS using two compartments. They report a first compartment accessible to dialysis of ∼4 L, a second non-accessible compartment of ∼10–16 L and inter-compartmental clearances of ∼90 mL/min. Employing these distribution values in a two-compartment model did not significantly change the predicted differences in plasma levels.

RESULTS

Fourteen patients maintained on thrice-weekly in-center nocturnal hemodialysis completed the two experimental periods consisting of seven hemodialysis treatments separated by a washout period. Patients' baseline characteristics are

summarized in Table 2. Their average age was 57 ± 10 years, and they had been maintained on hemodialysis for 3.9 ± 2.4 years and in-center nocturnal hemodialysis for 2.0 ± 1.5 years. Their routine dialysis prescriptions included an average blood flow of 304 ± 13 mL/min, a dialysate flow of 477 ± 26 mL/min and several non-reuse dialyzers. Treatment duration ranged from 6 to 8 h, providing average spKt/V_{urea} values well above current target guidelines at 2.6 ± 0.7. The dialysis prescriptions and values for spKt/V_{urea}, hemoglobin, albumin and phosphate levels were similar at the start of each experimental period, as summarized in Supplementary data, Table S1.

The prescriptions for the two experimental periods were designed to achieve widely different clearances for bound solutes while keeping the clearance for urea nearly the same, as summarized in Table 1. Dialytic clearances for urea, PCS and IS measured during the last treatment of each period are summarized in Table 3. In accord with the experimental design, the urea clearances were similar during the two periods. The lower blood flow employed during Period B prevented an increase in urea clearance, which would have occurred with the higher dialysate flow and larger dialyzer size.

In striking contrast, the dialytic clearances for the bound solutes were much higher during Period B than Period A. Clearances for these solutes were calculated in terms of both the total and free solute levels. For PCS, the total clearance 23 ± 4 mL/min in Period B was nearly double the total clearance of 12 ± 3 mL/min in Period A. Likewise, for IS, the total clearance was 30 ± 5 mL/min in Period B and 17 ± 3 mL/min in Period A. The free clearances for both solutes were also much higher in Period B than Period A (PCS: 548 ± 101 mL/min in Period B and 229 ± 49 mL/min in Period A; IS: 558 ± 117 mL/min in Period B and 249 ± 65 mL/min in Period A). For both solutes, the free clearances rose to a greater degree than the total clearances, reflecting significantly greater protein binding at the end of Period B than Period A.

The effects of the experimental dialysis prescriptions on plasma solute levels and solute removal are summarized in Table 4. Plasma urea levels were similar at the beginning of each period and did not change at the end of the periods. The amount of urea removed in the dialysate was also similar at the end of the two periods. The total and free plasma levels for PCS were also similar at the beginning of each period. Remarkably, the higher clearance provided in Period B did not have a different effect on total and free PCS levels than the lower clearance provided in Period A. The failure of increased clearance to reduce the PCS plasma levels was accompanied by a large increase in the amount of PCS removed in the dialysate at the end of Period B compared with Period A, as depicted in Figure 1.

In contrast, the higher clearance provided in Period B reduced the total and free plasma IS levels compared with Period A. The total and free plasma IS levels were similar at the beginning of each period. At the end of the higher-clearance Period B, the total level decreased by −4 (−17, +1)% and the free level by −11 (−39, −2)%, while at the end of the lower-clearance Period

Table 3. Solute clearances by dialysis

		A	B	P-value
Urea	Clearance (mL/min)	211 ± 42	222 ± 17	0.35
PCS	Total clearance (mL/min)	12 ± 3	23 ± 4	<0.001
	Free clearance (mL/min)	229 ± 49	548 ± 101	<0.001
	Pre-dialysis percent binding (%)	94.3 ± 1.5	95.4 ± 0.9	0.002
IS	Total clearance (mL/min)	17 ± 4	30 ± 5	<0.001
	Free clearance (mL/min)	249 ± 65	558 ± 117	<0.001
	Pre-dialysis percent binding (%)	93.0 ± 2.0	94.3 ± 1.2	0.007

Results are mean ± standard deviation. Dialytic clearances and percentage of solute binding were measured during the last treatment of each period. For PCS and IS, both total and free clearance values are reported. The total clearance represents the clearance expressed in terms of the total level, and the free clearance represents the clearance expressed in terms of the free, unbound level. Comparisons were performed using the paired *t*-test.

Table 4. Solute levels and dialytic removal

			A	B	P-value
Urea		Start level (mg/dL)	42 (38, 54)	42 (36, 46)	0.46
		End level (mg/dL)	45 (35, 52)	44 (39, 48)	
	% Change end versus start level		2 (−7, 18)	8 (1, 12)	0.49
		Amount removed in dialysate (g)	20 (18, 24)	21 (18, 23)	0.85
PCS	Total	Start level (mg/dL)	4.1 (3.1, 4.8)	3.2 (2.6, 4.0)	0.19
		End level (mg/dL)	4.1 (2.5, 4.5)	3.8 (3.5, 4.0)	
		% Change end versus start level	−8 (−18, 32)	11 (−5, 37)	0.50
	Free	Start level (mg/dL)	0.20 (0.16, 0.23)	0.16 (0.12, 0.21)	0.64
		End level (mg/dL)	0.23 (0.12, 0.27)	0.18 (0.14, 0.22)	
		% Change end versus start level	5 (−17, 84)	4 (−16, 31)	0.18
		Amount removed in dialysate (mg)	199 (111, 232)	269 (206, 312)	<0.001
IS	Total	Start level (mg/dL)	1.9 (1.0, 2.3)	2.1 (1.4, 2.4)	0.08
		End level (mg/dL)	2.2 (1.1, 2.9)	1.8 (0.9, 2.5)	
		% Change end versus start level	22 (14, 31)	−4 (−17, 1)	<0.001
	Free	Start level (mg/dL)	0.13 (0.06, 0.17)	0.12 (0.09, 0.16)	0.42
		End level (mg/dL)	0.14 (0.09, 0.27)	0.13 (0.06, 0.15)	
		% Change end versus start level	50 (7, 94)	−11 (−39, −2)	<0.001
		Amount removed in dialysate (mg)	116 (89, 170)	140 (87, 196)	0.15

Results are median (25^{th}, 75^{th} percentile). Start level was obtained pre-treatment at the first session of each period, and the end level was obtained pre-treatment at the last session of each period. The % change end versus start level refers to the change in levels from the start to end of each period. For the bound solutes, both the total and free levels are reported. Free levels of IS for one subject were omitted from the analysis because they were below the assay range. The amount removed in the dialysate was measured during the last treatment of each period. The effect of the experimental prescriptions on solute levels was analyzed using repeated measures ANOVA. Start levels and the amount removed in dialysate between Periods A and B were compared using the paired *t*-test.

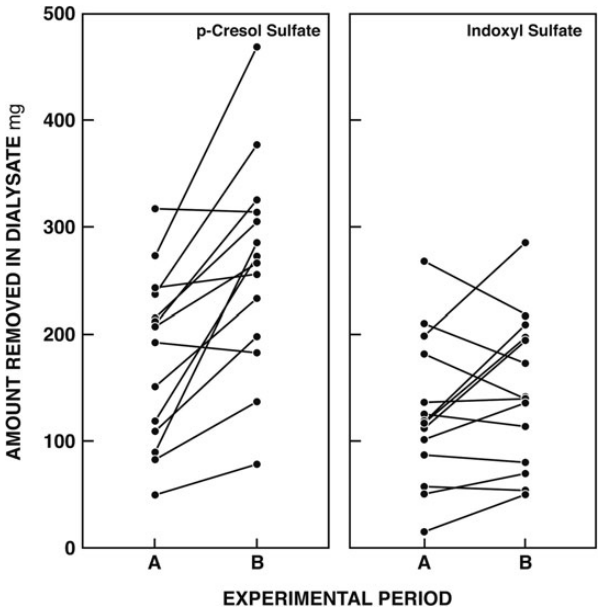

FIGURE 1: The dialytic removal of PCS and IS at the end of the two experimental periods. The amount of solute collected in the dialysate during the last session of each seven-treatment experimental period is plotted on the *Y*-axis. The dialytic removal for PCS (left panel) was significantly greater at the end of Period B compared with Period A. In contrast, the dialytic removal for IS (right panel) was not different at the end of the two periods.

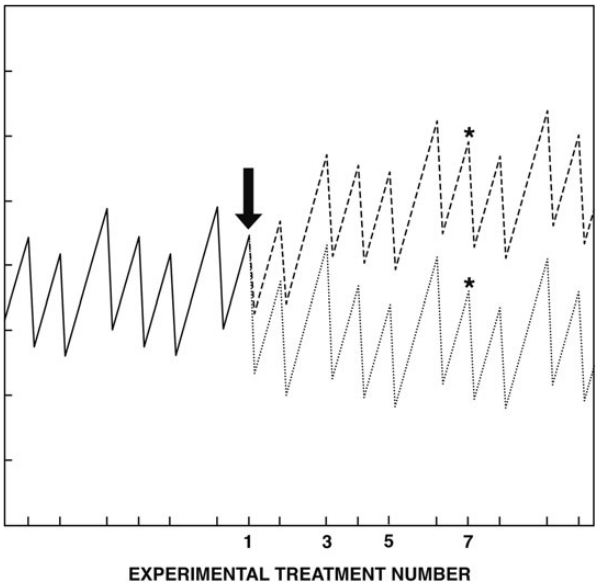

FIGURE 2: Predicted response of plasma PCS levels to a change in dialytic clearance if generation remained constant. Plasma levels in arbitrary units are plotted on the *Y*-axis versus the number of experimental treatments on the *X*-axis. Plasma levels during routine treatment prior to the experimental periods are represented by the solid line with the high-clearance experimental period represented by the dotted line and the low-clearance experimental period represented by the dashed line. The block arrow indicates the first experimental treatment, and the asterisks indicate the seventh treatment of each experimental period at which samples were collected. Concentrations were modeled as described in the Materials and methods using PCS clearances and an estimated volume of distribution of 15 L measured at the end of the experimental periods. Because we did not measure clearances during the baseline period, we used the average clearance from Periods A and B as the baseline clearance. Using different estimated baseline clearances did not significantly change the predicted difference at the end of the experimental periods, as illustrated in Supplementary data, Figure S2.

A, the total level increased by +22 (+14, +31)% and the free level by +50 (+7, +94)% (median, 25th, 75th percentile). Again in contrast to the finding with PCS, the average amount of IS removed in the dialysate was not different at the end of the two periods, as depicted in Figure 1. The normalized protein catabolic rate (nPCR) and albumin and phosphate levels were not different at the end of the two experimental periods, as summarized in Supplementary data, Table S2.

DISCUSSION

Protein-bound solutes are poorly cleared by conventional hemodialysis [14–16]. The two most extensively studied bound solutes, PCS and IS, have been associated with increased mortality and other adverse outcomes [3–7]. The dialytic clearance of bound solutes is limited because only the free, unbound portion can diffuse through the dialyzer membrane [2, 8]. In the native kidney, high clearances of bound solutes are achieved by tubular secretion, but this process is not replicated by hemodialysis [12]. As a result, these solutes may accumulate in the plasma of hemodialysis patients to a greater degree than urea.

Efforts have been made to increase the dialytic clearance of bound solutes with the goal of reducing their plasma levels [2, 16]. Two studies have assessed addition of convective to dialytic clearance [17, 18]. A crossover study found that 2 weeks of thrice-weekly hemodiafiltration with 60 L replacement volume reduced PCS plasma levels by ∼17% compared with conventional hemodialysis, while hemodiafiltration with 20 L replacement volume had no effect [17]. Another study found that 9 weeks of thrice-weekly hemodiafiltration with 19 L replacement volume lowered plasma PCS by ∼20% when compared with baseline levels [18]. Other studies have demonstrated that bound solute clearance can be increased by exposing plasma to sorbents, by addition of competitive inhibitors and by increasing plasma sodium concentration [19–23]. The effect of increasing clearance by these methods on bound solute plasma levels in humans, however, has not yet been reported.

We previously showed that the clearance of bound solutes could be increased by increasing dialysate flow and dialyzer size [8]. In patients receiving conventional thrice-weekly short treatment, connection of two dialysis machines in series was required to increase the dialysate flow sufficiently to achieve a large increase in bound solute clearances [9]. Studies in patients receiving thrice-weekly in-center nocturnal hemodialysis, however, allowed us to vary the dialysate flow over a wide range without having to add a second machine. Because such patients receive 6–8 h of dialysis per treatment, target Kt/V_{urea} values are easily achieved with relatively low dialysate flows and small dialyzers [10]. The effect of wide variations in dialysate flow and dialyzer size can therefore be assessed using standard equipment [11].

This study took advantage of our ability to vary dialysate flow and dialyzer size in nocturnal dialysis patients to test the extent to which sustained differences in clearance would alter the plasma levels of PCS and IS. Remarkably, a sustained difference in clearance did not lead to a difference in the plasma levels of PCS. The higher clearance was instead accompanied by a greater removal of PCS in the dialysate.

The simplest potential explanation for the failure of plasma PCS levels to respond to differences in clearance was that the experimental periods were too short. Modeling of the effects of a change in clearance convinces us that this was not the case. Assuming solute distribution in a single compartment of 13–16 L, we would predict that after six experimental treatments, plasma PCS concentrations would be within 5% of their ultimate equilibrium values if solute generation remained constant, as illustrated in Supplementary data, Figure S1. We would thus predict that if solute generation remained constant, the total plasma PCS levels would have been ∼40% lower at the end of Period B than Period A in the current study, as illustrated in Figure 2.

We should note, however, that previous studies have suggested that the dialytic behavior of PCS cannot be accounted for by distribution in a single compartment. Meijers *et al.* [24] observed lower reduction ratios during the second half of extended duration dialysis for PCS, and we subsequently observed the same phenomenon [11]. Eloot *et al.* [13] have recently analyzed the dialytic removal of PCS using a two-compartment model. Fitting the decline of plasma levels observed during standard hemodialysis treatments was optimized assuming a first compartment accessible to dialysis with a volume of ∼4 L, a second non-accessible compartment with a volume of ∼10 L and an inter-compartmental clearance of ∼90 mL/min. Such compartmental distribution would not notably affect the predicted reduction in PCS levels over the 2-week experimental period. A non-accessible compartment of much larger volume, however, could significantly increase the amount of time required for an increase in clearance to reduce PCS levels.

The failure of an increased clearance to reduce PCS levels could alternatively reflect the presence of a non-dialytic clearance. A continuously operating non-dialytic clearance diminishes the effect of increasing the dialytic clearance on a solute's plasma level. The modest reduction in plasma beta-2 microglobulin levels in response to a large increase in clearance seen in the Hemodialysis (HEMO) study is an example of this phenomenon [25–27]. The non-dialytic clearance, however, must be a considerable portion of the dialytic clearance to prevent an increase in dialytic clearance from lowering plasma levels. Thus, a non-dialytic PCS clearance of the order of 10 mL/min would have to be present to account for the similarity of plasma PCS levels at the end of Periods A and B in the present study. In addition, a non-dialytic clearance of this magnitude would prevent plasma PCS levels from rising to ∼10 times normal as they do in hemodialysis patients [1, 12]. In addition, Poesen *et al.* [28] observed that PCS plasma levels increased in proportion to the estimated glomerular filtration rate (eGFR) decline in chronic kidney disease, while urinary PCS excretion remained stable. This suggests that little PCS is cleared outside the kidneys so that there would be little non-dialytic clearance in our patients who had minimal residual kidney function.

If there is indeed little non-dialytic solute clearance, failure of solute levels to vary in proportion to sustained changes in dialytic clearance must be attributed to offsetting changes in solute generation. We thus presume that the 2-fold higher recovery of PCS from the dialysate at the end of Period B in the current study reflected a similar increase in PCS generation. Failure of PCS levels to fall with increased intensity of dialysis has recently been described in HEMO [25] study subjects randomized to treatments providing Kt/V_{urea} 1.7 or 1.3 three times per week [29]. PCS levels also failed to fall in proportion to increases in clearance in a previous study comparing hemodiafiltration with hemodialysis [17]. We suspect that the failure of PCS levels to respond in these situations was a consequence of increased generation. We can only speculate, however, as to the biochemical changes responsible for such variation in PCS generation. PCS is the sulfate conjugate of p-cresol, which is produced by colon microbes from phenylalanine and tyrosine [30–33]. The difference we observed in PCS generation cannot be ascribed to a difference in protein intake since plasma urea levels and urea removal rates were similar at the end of our two periods. A shift to sulfate conjugation of the minor portion of p-cresol, which is normally conjugated with glucuronide, could also not explain a large increase in PCS generation at the end of Period B [34, 35]. We thus assume that the difference in PCS generation reflected a shift in colon bacterial metabolism.

While plasma levels of PCS did not respond to a sustained difference in clearance, higher-clearance treatment did lower plasma levels of IS. This difference is not surprising given that p-cresol and indole are produced by different colon microbial enzymes and that the rates of PCS and IS generation are not closely correlated in individual subjects [28, 36, 37]. We should note, however, that a much larger study would be required to confirm that IS generation is independent of changes in clearance over the range studied here. In addition, studies in peritoneal dialysis patients have suggested that the generation of both PCS and IS is reduced when the dialytic clearance of these solutes is low [38].

Our findings suggest that for PCS, control of plasma solute levels may be achieved more effectively by suppressing generation than by increasing dialytic clearance. Reduction of PCS generation could potentially be achieved by manipulation of the colon microbiome [30, 31]. Microbial solute generation has been reduced by maneuvers including provision of dietary fiber, adsorbents and probiotics [32, 39–45]. Studies using DNA profiling have begun to characterize the composition of the microbiome in dialysis patients, and more sophisticated manipulations including targeted removal or addition of selected microbial enzymatic pathways may be possible in the future [39, 46].

Our study has limitations. First, the study was small, and solute levels can vary with time independent of the dialysis prescription. However, analysis of the predicted change in levels and the variability of the observed change in levels revealed a very low probability of failing to detect an effect of the experimental periods, as summarized in Supplementary data, Table S3. Second, we measured the effect of altered clearance on only two bound solutes. Many other bound solutes have been described and more probably remain to be identified [1]. Of note, both PCS and IS were more probably bound at the end of the high-clearance period when compared with the

low-clearance period. This finding suggests that levels of some other solutes that compete for binding had been reduced, but we can provide no information as to the identity of such solutes. Levels of bound solutes other than those we measured may be reduced more effectively by increasing the dialytic clearance. In addition, unrecorded changes to diet and medications may alter the generation of colon-derived solutes.

In conclusion, selectively changing the dialytic clearance of protein-bound solutes did not alter the plasma levels of PCS. The failure to reduce plasma levels may be explained by an increase in solute generation in response to the higher clearance, but further studies are needed to confirm this possibility. Suppression of solute generation may be required to reduce plasma PCS levels in dialysis patients.

SUPPLEMENTARY DATA

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

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CONFLICT OF INTEREST STATEMENT

None declared. Part of this work was presented in abstract form at the American Society of Nephrology Kidney Week in November 2014 in Philadelphia, PA.

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