Characteristics of Colon-Derived Uremic Solutes

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Abstract

Background and objectives Colon microbial metabolism produces solutes that are normally excreted in the urine and accumulate in the plasma when the kidneys fail. This study sought to further identify and characterize human colon-derived uremic solutes.

Design, setting, participants, & measurements Colon-derived solutes normally excreted in the urine were identified by comparing urine from controls (n=17) and patients with total colectomies (n=12), using an established metabolomic platform. Colon-derived solutes that accumulate in kidney failure were then identified by comparing the plasma of the control patients with that of patients on dialysis (n=14).

Results Ninety-one urinary solutes were classified as colon-derived on the basis of the finding of a urine excretion rate at least four-fold higher in control patients than in patients with total colectomies. Forty-six were solutes with known chemical structure, 35 of which had not previously been identified as colon-derived. Sixty of the colon-derived solutes accumulated in the plasma of patients with ESKD to a degree greater than urea and were therefore classified as uremic. The estimated urinary clearance for 27 out of the 32 colon-derived solutes for which clearance could be calculated exceeded that of creatinine, consistent with tubular secretion. Sulfatase treatment revealed that 42 out of the 91 colon-derived solutes detected were likely conjugates.

Conclusions Metabolomic analysis identified numerous colon-derived solutes that are normally excreted in human urine. Clearance by tubular secretion limits plasma levels of many colon-derived solutes.

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Introduction

Solutes normally excreted by the kidneys accumulate in CKD and cause uremic illness. Some of these uremic solutes are derived from colon microbes (1–6). Relatively few colon-derived uremic solutes have so far been chemically identified in humans. Attention has been concentrated on indoxyl sulfate, *p*-cresol sulfate, and tri-methylamine N-oxide, with substantial evidence for toxicity (7–9). These extensively studied solutes, however, are likely members of a much larger group.

This study sought to further characterize colon-derived solutes that accumulate in human kidney failure. Colon-derived solutes normally excreted by the kidneys were identified by comparing urine from control patients and patients who had undergone surgical colectomy. Comparison of plasma samples from the control patients and from patients undergoing maintenance hemodialysis then revealed the extent to which the colon-derived solutes accumulate in the plasma when the kidneys fail. Analysis of the plasma and urine of control patients allowed estimation of the efficiency with which the colon-derived solutes are normally cleared by the kidney. Use of an established metabolomic platform allowed chemical identification of an increased number of colon-derived solutes, along with detection of additional colon-derived solutes that have been repeatedly found in biologic samples but for which the chemical structure is not known. Treatment of urine with sulfatase tested whether the solutes without known chemical structure are conjugates.

Materials and Methods

Spot urine samples were collected from 17 patients with total colectomies. Colectomy patients were recruited if they had no active bowel disease, an eGFR >45 ml/min per 1.73 m^2, a serum albumin above 3 g/dl, no weight loss over 5% in the past 6 months, and no use of antibiotics in the past month. In 12 patients, the small intestine drained through an ileostomy without any ileal pouch, and in the other five patients, an ileal pouch had been created to allow control of defecation. Simultaneous spot urine and plasma samples were collected from 17 age-matched control patients who had no known gastrointestinal or kidney disease and no use of antibiotics in the last month. Pretreatment plasma samples were also collected at the midweek treatment from 14 patients maintained on hemodialysis who had negligible residual urine output, no history of gastrointestinal disease, and no use of antibiotics in the past month. The study was approved by the Stanford Institutional Review Board and was conducted in accordance with the Declaration of Helsinki.

Sample Preparation and Analysis

Daily creatinine excretion was estimated using the formulas of Cockcroft and Gault (10) and urine samples were diluted with water to achieve the creatinine concentration expected if the urine flow had been 40 ml/min. This entailed dilution of individual samples over a range from 15 to 227-fold. The intent of this step was to obtain peak areas in urine closer to those in plasma ultrafiltrate and to limit errors in mass spectrometric estimations of urine concentration arising simply from variability of urine flow rate. A subset of six samples were also diluted to achieve the creatinine concentration expected if the urine flow had been 10 ml/min and 0.6 ml aliquots of these samples were treated with 100 U sulfatase (S0626; Sigma) in sodium acetate buffer pH6 or buffer alone for 6 hours at 37°C before analysis. Plasma ultrafiltrate was prepared using Nanosep 30 separators and concentrated four-fold by drying and resuspension in water.

Metabolomic analysis was performed by Metabolon, Inc., using a liquid chromatography–mass spectrometry platform to identify solutes and estimate their relative concentrations (11,12). Metabolites were identified by comparing masses, retention times, and fragmentation patterns with a chemical reference library including over 4000 chemically confirmed metabolites as well as unnamed metabolites without confirmed chemical structure that have been repeatedly detected by Metabolon in biologic samples (11–13). Absolute concentrations were also measured for urea using an enzymatic method, for creatinine using HPLC, and for selected organic anions using liquid chromatography–tandem mass spectrometry with isotopic dilution as previously described (14,15).

Calculations and Statistical Analyses

Relative urinary excretion rates were estimated from solute peak areas measured in samples diluted to provide a uniform estimated urine flow. Estimated excretion rates were normalized to a body surface area of 1.73 m^2 using the formula of Mosteller (16). When no peak was detected for a given solute, a value equal to half of the smallest peak area detected in any sample of the same fluid type was imputed. Solutes were classified as colon-derived if their mean excretion rate was at least four-fold higher in individuals with intact colons than in those with total colectomies without ileal pouch and if the false discovery rate (q value) for the difference was <0.05. Solutes were classified as uremic if their mean peak area in plasma ultrafiltrate was at least 2.4-fold higher in patients on dialysis than the control patients, and the false discovery rate for difference was <0.05. The value of 2.4 was chosen because this was the ratio of urea peak areas in patients on dialysis relative to control patients.

The urinary clearance rate relative to creatinine was estimated by comparing peak areas in plasma ultrafiltrate and urine samples. The free, unbound fraction of solutes in the plasma was estimated by comparing peak areas in plasma ultrafiltrate and total plasma. Urinary clearance rates and free solute fractions were reported only when peak areas were measured in the urine, plasma and plasma ultrafiltrate of at least eight control patients. These calculations are on the basis of ratios of peak areas measured in different matrices and can provide only estimates of kidney clearance and protein binding. Conjugation of solutes without known chemical structure was assessed by comparing peak areas in urine samples treated with snail intestine sulfatase, which cleaves both sulfate and glucuronide conjugates, and with buffer control. Solutes without known chemical structure were classified as conjugates if they were detected in at least three of six urine samples and if sulfatase treatment reduced their peak area by an average of $>80\%$. These criteria were chosen because they identified urinary solutes with known chemical structure as either sulfate or glucuronide conjugates, with a sensitivity of 70% and specificity of 97% (Supplemental Table 1).

P values were calculated using the Wilcoxon rank-sum test to compare mass spectrometry peak areas and the Pearson chi-squared test to compare the proportions of colon-derived and noncolon-derived solutes with specified characteristics. P values were obtained using Stata release 11 and false discovery rates (q-values) were calculated using software from http://qvalue.princeton.edu. This procedure identifies significant differences among a large number of comparisons at the expense of labeling a predetermined proportion of these comparisons (here, 0.05) as significant by chance (17).

Results

Characteristics of the study patients are summarized in Table 1 and further detailed in Supplemental Tables 2 and 3. The patients with colectomy appeared well nourished, with average body mass index of 26 kg/m^2 and serum albumin of 3.7 g/dl. Their eGFR was similar to that of the control patients. Colectomy was performed for inflammatory bowel disease in nine out of 12 patients who had colectomies without ileal pouches and all five patients who had colectomies with ileal pouches.

A total of 855 solutes were detected in the urine of control patients with intact colons. Of these, 464 were named solutes with known chemical structure and 391 were without known chemical structure. Ninety-one of these 855 urinary solutes were classified as colon-derived, including 46 with known chemical structure (Table 2) and 45 without known chemical structure (Supplemental Table 4). A careful literature search (Supplemental Table 5) revealed a known colonic origin for only the minority of the 46 colon-derived solutes with known chemical structure identified in this study. In contrast to the large number of solutes identified as colon-derived, none of the remaining 764 solutes had a four-fold higher urinary excretion rate in patients with colectomy compared with control patients, with a q value <0.05 for this difference.

A total of 880 solutes were detected in the pretreatment plasma samples of patients on maintenance hemodialysis. On the basis of comparison of peak areas in the plasma ultrafiltrate of patients on hemodialysis and control patients, 492 of these solutes were classified as uremic, including 278 with known chemical structure and 214 without known chemical structure (Supplemental Table 6). Solute levels in individual participants exhibited wide variability (Supplemental Table 7). The majority of the 91 colon-derived solutes excreted in the urine of control patients were classified as uremic, including 33 with known chemical structure (Table 2) and 27 without known chemical structure (Supplemental Table 4). Nineteen colon-derived solutes normally excreted in the urine could not be categorized because they were not detected in plasma

Table 1. Patient characteristics

Characteristics	Colectomy without Ileal Pouch (*n*=12)	Colectomy with Ileal Pouch (*n*=5)	Intact Colon (*n*=17)	Hemodialysis (*n*=14)
Age, yr	50±17	51±15	50±14	54±14
Female, %	50	0	35	21
Body mass index, kg/m^2	26±7	27±6	24±4	24±3
eGFR, ml/min per 1.73 m^2	93±28	93±29	86±17	0
Diabetes, %	0	0	0	64[a]
Immunosuppressive medications, *n*	2/12	1/5	0	0

Values are mean±SD unless otherwise stated. eGFR estimated by the CKD Epidemiology Collaboration equation. Colectomy without ileal pouch was performed for Crohn disease (5), ulcerative colitis (4), colonic inertia (1), ischemia (1) and familial adenomatous polyposis (1). Colectomy with ileal pouch was performed for Crohn disease (2) and ulcerative colitis (3).
[a]$P<0.05$ intact colon versus hemodialysis; differences between patients with colectomy without ileal pouch and patients with intact colon were not significant.

ultrafiltrate and 12 others were not classified as uremic on the basis of their relative peak areas in plasma ultrafiltrate from patients on dialysis and control patients.

Simultaneous analysis of urine and plasma ultrafiltrate permitted estimation of the urinary clearance relative to creatinine for some of the colon-derived solutes (Supplemental Table 4, Table 2). Of note, the majority these solutes had a clearance more than two-fold higher than that of creatinine consistent with tubular secretion. Secretory clearance of many colon-derived solutes was associated with binding to plasma proteins (Table 3). Values for clearance and protein binding are on the basis of ratios of peak areas measured in different matrices and therefore can only be considered to be estimates. Measurements using quantitative assays with chemical standards largely confirmed the results obtained by metabolomic analysis (Table 4). In particular, these measurements suggested that metabolomic analysis had not overestimated the extent of microbial solute production or of solute accumulation in patients on dialysis. We have, however, previously observed discrepancies in the estimation of solute accumulation by metabolomic and quantitative analysis and suspect that metabolomic assessment of lower-abundance solutes may be particularly subject to error (18).

Nearly half of the colon-derived solutes with known chemical structure were sulfate or glucuronide conjugates. Sulfatase treatment reduced the chromatographic peak areas by >80% in 23 out of the 45 colon-derived urinary solutes without known chemical structure, suggesting that these were sulfate or glucuronide conjugates (Supplemental Table 1, Table 3). Fifteen out of the 27 colon-derived uremic solutes without known chemical structure were among these presumed conjugates (Supplemental Table 4).

The estimated urine excretion rate for the colon-derived solutes in the 12 patients with total colectomy without ileal pouch averaged 11%±7% of that in control patients. In comparison, the estimated urinary excretion rate for the colon-derived solutes in the five patients with total colectomy with ileal pouch averaged 47%±37% of that in control patients (Supplemental Table 8). Statistical comparison was limited by the small patient number and by missing values, but the estimated excretion rate for each of the 87 colon-derived solutes detected in colectomy urine samples was numerically higher in the patients with pouches.

Discussion

Colon-derived uremic solutes have attracted particular interest because their production could prove easier to suppress than production of uremic solutes derived from mammalian metabolism (1–6). Despite this interest, relatively few colon-derived uremic solutes have so far been chemically identified in humans (Supplemental Table 5) (19–21). Examination of a small group of patients on dialysis with surgical colectomies suggested that the number of colon-derived uremic solutes in humans is large, but identified only six solutes (19). Many more colon-derived solutes have been identified in rats and mice in which suppression of microbial solute production is easier to accomplish. Studies in these species have identified at least 27 colon-derived solutes which are normally excreted in the urine and/or accumulate in the plasma when kidney function is reduced (Supplemental Table 5) (1,22–28).

This study greatly expands the list of colon-derived solutes known to accumulate in human kidney failure. We identified 33 such solutes, of which 22 had not been previously identified as colon-derived. It is important to emphasize that the number of solutes detected depends on the analytic method. We used the largest widely available metabolomic platform but it by no means detects the full array of solutes made by microbes (29). We also found that 27 solutes in the Metabolon database without known chemical identity are colon-derived solutes that accumulate in kidney failure. We suspect the total number of such solutes is in fact considerably larger than reported here, as no current analytic platform is capable of detecting the full spectrum of solutes in the human metabolome (11,18). Thirteen solutes previously identified as colon-derived uremic solutes were not identified as such in this study, as described in detail in Supplemental Table 9.

Most of the colon-derived solutes we identified had kidney clearances higher than that of creatinine. We presume these high clearances are achieved by secretory mechanisms that have been localized largely in the proximal tubule (30). Seven of the colon-derived uremic solutes are known substrates of proximal tubular organic anion transporters OAT1 and OAT3 (Supplemental Table 10) (12,31,32), including 2-oxindole-3-acetate and catechol sulfate, which have not previously been confirmed as

Table 2. Colon-derived solutes excreted in human urine

Colon-Derived Solute	Urinary Excretion Rate Colectomy/ Control	Detected in Control Patient Urine, %	Detected in Colectomy Urine, %	Previously Identified as Colon-Derived	Plasma Ultrafiltrate Hemodialysis/ Control	Urinary Clearance Rate Relative to Creatinine	Free Fraction, %
Identified as uremic							
Phenylacetylglutamate	0.18	100	92		319.2		
Cinnamoylglycine	0.08	100	67	x	177.3		
p-Cresol glucuronide	0.00	100	8	x	171.7	5.3±2.1	8±5
Phenylacetylthreonine	0.20	94	8		161.4		
Phenylacetylserine	0.14	82	17		126.6		
Phenylacetylalanine	0.14	100	25		125.3		
4-Acetylphenol sulfate	0.24	94	50		122.0		
Phenylacetylmethionine	0.12	82	0		114.5		
6-Hydroxyindole sulfate	0.08	100	33		83.4	18.8±7.2	1±0.6
Phenylacetylhistidine	0.11	100	25		82.5		
3-(3-Hydroxyphenyl) propanoic acid sulfate	0.04	100	8		77.7		
Trimethylamine N-oxide	0.14	100	100	x	59.8	1.7±0.6	37±7
Indoxyl sulfate	0.08	100	100	x	52.6	18.7±4.6	2±0.7
Phenylacetylglutamine	0.22	100	100	x	46.6	3.6±0.6	37±5
2-Oxindole-3-acetate	0.13	100	58		38.6		
3-Hydroxyhippuric acid	0.04	100	67	x	30.6	13.2±8.6	25±8
p-Cresol sulfate	0.01	100	100	x	27.4	7±1.2	2±0.4
3-Methoxycatechol sulfate	0.19	100	100		25.4	4.9±1.3	8±2
Thioproline	0.25	76	17		22.7	0.6±0.5	3±1
Indoleacetic acid	0.11	100	100		18.1	8.8±8.2	2±0.8
2,8-Quinolinediol sulfate	0.07	88	25		17.4		
4-Ethylphenylsulfate	0.01	100	50	x	16.6		
Phenol sulfate	0.23	100	100	x	16.6	2.4±0.4	7±2
Vanillyl alcohol sulfate	0.13	100	83		14.2	8.1±12.6	46±57
2-Acetamidophenol sulfate	0.04	100	17		12.5		
2-Aminophenol sulfate	0.12	100	75	x	11.3	0.9±0.3	29±18
4-Methylcatechol sulfate	0.04	100	67	x	11.1	8.4±2.9	3±1
Formylanthranilic acid	0.20	100	42		8.1		
Azelaic acid	0.22	100	67		6.3	1.7±1.9	60±33
Pyrocatechol sulfate	0.23	100	92		5.1	3.2±0.9	9±2
Gentisic acid	0.10	100	67		4.8		
1,2,3-Benzenetriol sulfate[a]	0.17	100	92		4.0		
5-Hydroxyhexanoaic acid	0.12	65	8		2.5	0.7±0.3	64±49
Not identified as uremic							
CMPF	0.19	59	8		2.6		
3-(3-Hydroxyphenyl) propanoic acid	0.22	59	0		2.1	1.4±0.9	11±3
5-Androstenediol disulfate	0.15	94	50		1.5		
Picolinic acid	0.16	88	25		1.3	0.7±0.3	25±7
N-Methyltaurine	0.07	88	0		1.3		
Fructose	0.20	82	42		0.8	0.03±0.02	110±49
1,2,3-Benzenetriol sulfate[b]	0.11	88	58				
3-Hydroxyphenylacetatic acid	0.11	100	33				
Indolepropionylglycine	0.04	100	8				
N-Acetylhistamine	0.18	100	75				
Piperidine	0.07	100	100				
Pregnen-diol disulfate	0.21	100	75				
Triethanolamine	0.14	100	92				

Values are mean±SD unless otherwise stated. Urinary clearance rate relative to creatinine and free fraction are reported if both values could be calculated for at least eight out of 17 control patients. Clearance rates and free fractions are calculated from peak areas measured in different sample matrices and provide only estimates of the extent of secretion and protein binding. Solutes were classified as uremic if the ratio of average plasma ultrafiltrate hemodialysis to average control peak areas was >2.4 with the difference in average peak areas $q<0.05$. Compound names are those used by the Human Metabolomic Database (47), except for CMPF, which is 3-carboxy-4-methyl-5-propyl-2-furanpropanoate.

[a]Sulfate group on second carbon in 1,2,3-benzenetriol sulfate.

[b]Sulfate group on first carbon in 1,2,3-benzenetriol sulfate.

Table 3. Characteristics of colon-derived solutes

Characteristic	Colon-Derived Solutes	Noncolon-Derived Solutes
Ratio of solute clearance to creatinine clearance in the native kidney of >2	24/32 (75%)[a]	114/367 (31%)
Free fraction <10%	15/32 (47%)[a]	31/367 (8%)
Ratio of plasma ultrafiltrate level in patients on hemodialysis relative to control patients is >10	49/60 (82%)[a]	232/432 (54%)
Solutes with known chemical structure that are sulfate or glucuronide conjugates	18/46 (40%)[a]	36/383 (10%)
Solutes without known chemical structure presumed to be sulfate or glucuronide conjugates on the basis of results of sulfatase treatment	23/38 (61%)[a]	78/334 (23%)

[a]$P<0.001$ proportion of colon-derived solutes compared with noncolon-derived solutes. Clearance values and free fractions were compared for 399 solutes for which metabolomic peak areas were reported in both urine and plasma ultrafiltrate in at least eight out of 17 control patients. The ratio of average plasma ultrafiltrate levels in patients on dialysis and control patients was calculated for all of the 492 solutes classified as uremic. Solutes that are sulfate or glucuronide conjugates were identified by their chemical formula among 429 solutes with known chemical structure found in the urine of at least eight out of 17 control patients, and by sulfatase treatment among 372 solutes without known chemical structure found in at least three out of six treated urine samples, as described in the *Materials and Methods*.

colon-derived (Supplemental Table 5). For several of the colon-derived uremic solutes, binding to plasma proteins allowed estimated clearance rates expressed in terms of the free-solute concentration to exceed the estimated kidney plasma flow (Table 2). Such high clearance rates presumably serve to maintain the free solute levels of some toxic solutes very low. Our data suggest that a large portion of colon-derived solutes are protein bound and rapidly cleared by secretion (Table 3). The plasma levels of protein-bound secreted solutes tend to remain high in patients maintained on hemodialysis because the dialytic solute clearances are low relative to their clearances in the normal kidney (15). Indeed, the majority of the colon-derived urinary solutes are more than ten-fold elevated in the plasma ultrafiltrate of patients on hemodialysis compared with control patients (Table 3).

Almost half of the colon-derived uremic solutes with known chemical structure were sulfate or glucuronide conjugates and sulfatase treatment identified many of the unnamed colon-derived uremic solutes as likely conjugates. Conjugation in general is presumed to reduce toxicity and facilitate excretion (33). An additional 12 of the named colon-derived uremic solutes were amino-acid conjugates. Seven solutes were conjugates of amino acid and phenylacetic acid, which is produced by colon microbes from phenylalanine (21,34). One of these, phenylacetylglutamine, has been associated with cardiovascular disease and mortality in CKD (35). The finding of six additional amino-acid conjugates of phenylacetic acid suggests that molecules related to phenylacetylglutamine may accumulate in parallel. Phenylacetylmethionine and phenylacetylglutamate have been detected in human plasma and urine, respectively, but we did not find previous reports identifying the other conjugates (36,37). Of note, microbes can both oxidize and reduce amino acids. Thus, although phenylacetic acid is produced by oxidative metabolism of phenylalanine, cinnamoylglycine is produced by reductive metabolisms of the same amino acid. Similarly, *p*-cresol sulfate is produced by oxidative metabolism of tyrosine whereas 3-(3-Hydroxyphenyl) propanoic acid sulfate and 3-Hydroxyhippuric acid are produced by reductive metabolism of tyrosine.

Table 4. Measurements by metabolomic and quantitative assays

Solute	Metabolomic				Quantitative			
	Urinary Excretion Rate Colectomy/Control	Hemodialysis/Control	Urinary Clearance Rate Relative to Creatinine	Free Fraction, %	Urinary Excretion Rate Colectomy/Control	Hemodialysis/Control	Urinary Clearance Rate Relative to Creatinine	Free Fraction, %
Urea	0.83	2.4	0.5±0.1	75±11	0.86	3.1	0.6±0.1	—
Creatinine	—	3.6	—	50±5	—	11.6	—	—
Indoxyl sulfate	0.08	52.6	18.7±4.6	2±0.7	0.08	144.5	28.4±6.9	2±0.4
p-Cresol sulfate	0.01	27.4	7±1.2	2±0.4	0.00	45.4	9.6±2.5	2±0.4
Phenylacetylglutamine	0.22	46.6	3.6±0.6	37±5	0.12	103.2	4.1±0.7	114±17
Hippurate	0.54	36.7	12±5.2	31±6	0.81	87.9	11.6±5.3	33±3

Values are mean±SD unless otherwise stated. Metabolomic values are calculated from peak areas and quantitative values are calculated from absolute solute concentrations measured on the same samples as described in the methods. Hemodialysis/control is the ratio of average peak area or concentration in the ultrafiltrate of patients on hemodialysis relative to control patients.

Findings on some other solutes are worth noting. Indoleacetic acid has been shown to be prothrombotic in animals and to be independently associated with cardiovascular outcomes and mortality in patients with CKD (38,39). Fecal analysis has shown it is produced by colon microbes, but the extent to which its accumulation in host urine or plasma depends on microbial production has not been confirmed (12,40). Carboxy-4-methyl-5-propyl-2-furanpropionic acid (CMPF) has been associated with gestational diabetes and impaired insulin production (41). Microbial origin of CMPF has been suspected but has not previously been confirmed in humans (27). CMPF has been classified as a uremic solute in past studies and was 2.6-fold elevated in patients on hemodialysis in this analysis, but did not meet statistical criteria as a uremic solute as it was only detected in half of the hemodialysis plasma ultrafiltrate samples (42).

We presume that solutes we have identified as colon-derived have their origin in microbial metabolisms rather than colon cell metabolism. Urinary excretion of solutes identified as colon-derived was consistently higher in patients with colectomy with ileal pouches than in those without pouches. This presumably represents solute production by microbes colonizing the pouches (43,44). These pouches have a volume on the order of 250–500 ml and the end ileal fluid resides in them at body temperature for some time, allowing microbial growth and metabolism (45,46). Microbial colonization of the end-ileum has also been demonstrated in colectomy patients without ileal pouches, which may account for urinary excretion of small quantities of colon-derived solutes in these patients (25,44). In addition, some solutes may be generated by both microbial and mammalian metabolism with the predominant process dependent on diet and species. Hippurate, which was not classified as colon-derived in this study, exemplifies this possibility (1,19,25,27).

Our study has limitations. The cutoffs for classification of solutes as colon-derived and uremic were arbitrary. Use of different cutoff values would yield different numbers of solutes in each class (Supplemental Table 11). The extent to which solutes accumulate in patients on dialysis depends on their dialytic clearance, which was not measured in this study. An important limitation of metabolomic analysis is that in obtaining data on large numbers of solutes we sacrifice accuracy in the determination of their individual concentrations. Calculations of urinary clearance and plasma protein binding on the basis of ratios of mass spectrometric peak areas measured in different matrices provide only estimates of the extent of tubular secretion and protein binding. Imputation of minimum peak area values when solute levels are below the limits of detection can also introduce errors in classification. In this study, nonparametric statistical analysis would not allow classification of a solute as uremic if it were detected in the plasma ultrafiltrate of fewer than eight out of the 17 control patients. Similar limitations apply to the classification of solutes as colon-derived. The patient number was small, and colon-derived solutes that are produced in a minority of people would not have been detected. Differences in urine solute excretion in patients with colectomy could be related to characteristics other than the absence of colon microbes. There could, for instance, be differences in diet and the prior history of inflammatory bowel disease in many patients with colectomy could contribute to group differences in metabolism. An even greater limitation of this study, however, is that no current metabolomic platform detects the whole range of solutes produced by mammalian or microbial metabolism. We thus suspect the number of colon-derived uremic solutes identified in humans will continue to increase.

In summary, metabolomic profiling of individuals with colectomies and normal kidney function identified 91 urinary colon-derived solutes, including over 30 named solutes not previously shown to be colon-derived. Many of these urinary colon-derived solutes were protein bound and efficiently eliminated by the kidney through tubular secretion. Most of them were shown to accumulate in kidney failure. Sulfatase treatment identified many colon-derived solutes without known chemical structure as conjugates. The toxicity of most uremic colon-derived solutes remains to be studied.

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Disclosures

None.

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