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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Supplementary Materials

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Supplementary Table 1: Identification of solutes as sulfate or glucuronide conjugates by sulfatase treatment

	Peak Area Sulfatase/Buffer < 0.2
Urinary solutes with known chemical structure which are sulfate conjugates	46/64 (72%)
Urinary solutes with known chemical structure which are glucuronide conjugates	32/47 (68%)
Urinary solutes with known chemical structure which are neither glucuronide or sulfate conjugates	12/462 (3%)
Colon-derived urinary solutes without known chemical structure	23/38 (61%)

Results show the fraction of solutes measured in at least 3 of the 6 urine samples treated with sulfatase for which peak area was reduced by sulfatase treatment to less than 0.2- fold the peak area in samples treated with buffer alone. These criteria were chosen because they identified solutes with known chemical structure as sulfate or glucuronide conjugates with high sensitivity (70%) and specificity (97%). These criteria identified 23 of the 38 colon-derived solutes without known chemical structure which were detected in at least 3/6 urine samples treated with sulfatase as likely conjugates.

Supplementary Table 2: Details of colectomy surgeries

	Colectomy without Ileal Pouch (n=12)	Colectomy with Ileal Pouch (n=5)
Years Since Colectomy	5 ± 9	22 ± 15
Reason for Colectomy		
Crohn's Disease	5	2
Ulcerative Colitis	4	3
Familial Adenomatous Polyposis	1	
Colonic Inertia	1	
Ischemia	1	

Values are mean ± standard deviation

Supplementary Table 3: Details of hemodialysis patients

	Hemodialysis Patients (n=14)
single pool Kt/v urea	1.6 ± 0.3
Hours per Session	3.5 ± 0.4
Sessions per Week	3
Access Blood Flow (ml/minute)	410 ± 45
Dialysate Flow (ml/minute)	600 ± 136
Years on Hemodialysis	6.4 ± 2.9
Cause of ESRD	
Diabetes	9
Hypertension	4
Lupus	1

Values are mean ± standard deviation

ESRD: End stage renal disease

Supplementary Table 4: Characteristics of colon-derived solutes without known chemical structure

Metabolon ID	Neutral Mass	Urinary Excretion Rate Colectomy/ Control	Detected in Control Urine (%)	Detected in Colectomy Urine (%)	Plasma Ultrafiltrate Hemodialysis/ Control	Urinary Clearance Rate Relative to Creatinine	Free Fraction Control (%)
Identified as Uremic							
X – 13726	380.04219	0.17	47	0	531.8		
X – 21821	244.08558	0.05	100	8	222.8		
X – 17351	244.08518	0.05	100	8	164.1		
X – 12126	325.08118	0.07	94	8	157.0		
X – 11843	231.02026	0.03	94	8	117.7		
X – 22508	325.0805	0.04	100	17	95.5		
X – 12261	259.01527	0.10	59	0	94.9		
X – 12830	373.12005	0.16	88	0	91.4	48.8 ± 24.3	4 ± 2
X – 12718	325.08117	0.06	100	25	82.6		
X – 12013	243.02044	0.02	94	0	79.3		
X – 22509	474.15354	0.08	94	0	75.2		
X – 12216	229.00471	0.05	100	50	71.9	21.5 ± 10.8	16 ± 8
X – 17367	183.09017	0.06	94	33	50.6	10.1 ± 4.7	21 ± 4
X – 17354	474.15447	0.05	94	0	43.0		
X – 13729	242.98409	0.07	100	25	39.7	21.8 ± 11.3	11 ± 6
X – 21839	453.20165	0.17	53	0	39.4		
X – 24757	181.07375	0.08	94	33	26.2		
X – 17686	260.03572	0.24	94	58	26.1		
X – 12543	182.05826	0.01	100	83	22.3	9.1 ± 4.3	32 ± 10
X – 12283	244.08439	0.05	100	8	13.1		
X – 17692	371.10545	0.24	71	25	12.9		
X – 17325	185.10533	0.08	100	67	10.5	7.6 ± 5.1	34 ± 12

X – 21310	234.99186	0.08	100	33	7.6	19.1 ± 7.9	1 ± 0.8
X – 13866	254.11558	0.23	53	0	3.8		
X – 12212	230.02521	0.15	88	25	3.7		
X – 17438	246.14656	0.09	100	25	3.1		
X – 12740	288.03057	0.00	100	0	2.9	37.9 ± 56.1	9 ± 8
Not Identified as Uremic							
X – 23997	222.06717	0.01	100	0	17.1	20.2 ± 12.5	2 ± 0.9
X – 21845	357.12607	0.10	88	0	5.3		
X – 16071	145.05269	0.18	94	50	2.1	1.5 ± 0.8	2 ± 1
X – 23583	115.06341	0.11	94	0	1.7	2.5 ± 1.5	63 ± 28
X – 21258	214.03044	0.22	71	17	1.3		
X – 12815	272.03571	0.03	53	0	1.2		
X – 11640	378.0778	0.12	76	0			
X – 12027	243.02026	0.04	65	8			
X – 12306	248.03574	0.10	100	17		10.7 ± 5.5	10 ± 5
X – 15728	232.04068	0.08	82	0			
X – 17371	453.20085	0.11	76	0			
X – 17673	149.98072	0.05	71	25			
X – 21828	373.12114	0.10	82	0			
X – 23657	143.09444	0.03	94	50			
X – 24272	275.0093	0.09	71	0			
X – 24490	280.1058	0.07	100	100			
X – 24760	137.50476	0.08	82	17			
X - 24764	165.09982	0.16	100	75			

Values are mean ± standard deviation. Urinary clearance rate relative to creatinine and free fraction are reported if both values could be calculated for at least 8/17 control subjects. 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 plasma ultrafiltrate hemodialysis/ control was >2.4 with $q<0.05$. Solutes named according to format "X-12345" are metabolites in the Metabolon database that have been identified in past studies but do not have known chemical structure.

Supplementary Table 5: Previously identified colon-derived solutes. Solutes were considered to be previously identified as colon-derived if literature review identified a study reporting lower urine or plasma levels in animals or humans who were germ free, antibiotic treated, sorbent treated, or who had undergone surgical removal of the total colon.

First Author	Nicholls (1)	Claus (2)	Yap (3)	Wikoff (4)	Lee (5)	Kikuchi (6)	Mishima (7)	Kikuchi (8)	Jaganath (9)	Gonzalez-Barrio (10)	Stalmach (11)	Tang (12)	Aronov (13)	Tanaka (14)	Nazzal (15)	Present Study
Year	2003	2008	2008	2009	2012	2010	2017	2017	2006	2010	2013	2013	2011	2015	2017	
PMID	14615964	18854818	18698804	19234110	22300547	20870466	28396122	27701177	17015248	20218618	22961385	23614584	21784895	26317986	28379433	
Species	Rats	Mice	Mice	Mice	Rat	Rat	Mice	Rat	Human	Human	Human	Human	Human	Human	Human	
Intervention	Germ Free	Germ Free	Antibiotics	Germ Free	Antibiotics	AST-120	Germ Free	Colectomy	Colectomy	Colectomy	Colectomy	Antibiotics	Colectomy	Colectomy	Antibiotics	
Renal Function	Control	Control	Control	Control	Control	CKD	CKD	Control	Control	Control	Control	Control	ESRD	ESRD	ESRD	
Specimen	Urine	Urine	Urine	Plasma	Urine	Plasma and Urine	Plasma and Urine	Urine	Urine	Urine	Urine	Plasma and Urine	Plasma	Plasma	Plasma	
Hippurate	x	x	x	x	x	y	y	x								
Indoxyl sulfate				x		y	y	x					y	y	y	y
p-Cresol sulfate						y	y	x					y	y	y	y
TMAO	x						y					x				y
Phenylacetylglycine	x	x		x	x		y									
Phenol sulfate				x		y	y	x								y
Phenylacetylglutamine													y	y		y
3-Hydroxyhippuric acid											x			y		y
4-Ethylphenyl sulfate						y										y
Cholate							y									
N,N-Dimethylglycine							y									
Glutarate							y									
Serotonin				x												
Indole propionic acid				x												
Phenylpropionylglycine				x												
Cinnamoylglycine				x												y
Equol sulfate				x	x											
Methyl equol sulfate				x												
Uric acid				x												
Dihydroxyquinoline glucoronide				x												
12-Hydroxy-Eicosapentaenoic acid				x												
3-Hydroxycinnamic acid		x														
4-Hydroxypropionic acid		x														
N-Acetylated glycoprotein		x														
Indole-3-Carboxylic acid					x											
3-Methyldixoyindole					x											
Tryptophanol					x											
p-Cresol glucuronide					x											y

3-Mercaptolactate-Cysteine disulfide					x											
S-(Hydroxymethyl) glutathione					x											
7-Hydroxy-6-Methyl-8-Ribityl lumazine					x											
Taurochenodeoxycholate-7-Sulfate					x											
6-Hydroxy-5-Methoxyindole glucuronide					x											
3-Hydroxypropionic acid	x															
5-Hydroxyindole													y			
Indoxyl glucuronide													y			
3-Indolepropionic acid													x			
Phenylacetic acid														y		
2-Aminophenol sulfate														y		y
2-Methoxyphenol sulfate														y		
4-Methylcatechol sulfate														y		y
3-(3-(Sulfoxy)phenyl) propanoic acid														y		
3-Hydroxybenzoic acid											x					
3-(3-Hydroxyphenyl) Hydracrylic acid											x					
3-(3-Hydroxyphenyl) propionic acid											x					
3-(3,4-Dihydroxyphenyl) propionic acid											x					
2-Hydroxypentanoate							y									
4-Guanidinobutanoic acid (ᵞ-Guanidino butyrate)							y									
Succinate							y									
3,4-Dihydroxyphenylacetic acid									x							
3-Methoxy-4-Hydroxyphenylacetic acid									x							
3-Hydroxyphenylacetic acid									x							
Urolithin A-O-Glucuronide										x						
Urolithin B-O-Glucuronide										x						

x Decreased in germ free group

y Decreased in germ free group and increased in renal insufficiency

CMPF: 3-carboxy-4-methyl-5-propyl-2-furanpropanoate; TMAO: Trimethylamine N-oxide

Supplementary Table 6: Complete list of uremic solutes. Solutes considered uremic if ratio HD/Control > 2.4 and q<0.05. PMC 4552939 is the previously longest published list of uremic solutes.

Uremic Solute Metabolon Name	Neutral Mass	HD/Control	Cited in PMC 4552739 (14)
indoxyl glucuronide	309.08486	495.2	x
phenylacetylglutamate	265.09503	319.2	
1-methylguanidine	73.06398	285.6	x
methyl-4-hydroxybenzoate sulfate	232.00417	233.4	
cinnamoylglycine	205.0739	177.3	x
p-cresol-glucuronide	284.08961	171.7	x
4-hydroxyphenylacetylglutamine	280.10593	170.1	
gamma-CEHC glucuronide	440.16826	166.1	x
trizma acetate	121.07388	161.9	
phenylacetylthreonine	237.10012	161.4	
indoleacetylglutamine	303.12192	151.7	x
tartarate	150.01645	129.3	
4-ethylphenol glucuronide	298.10526	128.0	
phenylacetylserine	223.08447	126.6	
phenylacetylalanine	207.08955	125.3	
4-acetylphenol sulfate	216.00925	122.0	
phenylacetylmethionine	267.09292	114.5	
11-ketoetiocholanolone glucuronide	480.23594	113.8	
furaneol sulfate	208.00417	96.9	
3-hydroxyphenylacetate sulfate	232.00417	95.6	
hydroquinone sulfate	189.9936	84.2	x
6-hydroxyindole sulfate	213.00959	83.4	
phenylacetylhistidine	273.11133	82.5	
4-hydroxyhippurate	195.05317	82.5	x
phenol glucuronide	270.07396	79.3	x
3-(3-hydroxyphenyl)propionate sulfate	246.01982	77.7	
trans-2-hexenoylglycine	171.08955	72.1	
etiocholanolone glucuronide	466.25668	65.4	
trimethylamine N-oxide	75.0684	59.8	x
3-methylurate	182.044	59.7	
androsterone glucuronide	466.25668	59.3	
N-acetyl-3-methylhistidine	211.09568	59.3	x
vanillactate	212.06848	54.6	

3-acetylphenol sulfate	216.00925	54.2	
3-indoxyl sulfate	213.00959	52.6	x
4-methoxyphenol sulfate	204.00925	51.2	
kynurenate	189.0426	47.4	x
phenylacetylglutamine	264.11102	46.6	x
guaiacol sulfate	204.00925	45.4	
methylsuccinoylcarnitine	275.13688	43.1	
2-methylcitrate/homocitrate	206.04267	41.2	
3-methylglutarylcarnitine	289.15253	41.2	x
5-hydroxyindoleacetate	191.05825	40.8	x
7-methylurate	182.04398	40.4	x
syringol sulfate	234.01982	40.2	
eugenol sulfate	244.04055	39.6	x
2-oxindole-3-acetate	191.05825	38.6	
S-(3-hydroxypropyl)mercapturic acid (HPMA)	221.07219	37.8	
hippurate	179.05825	36.7	x
N-acetylkynurenine	250.09537	34.8	
phenylacetylglycine	193.0739	33.8	x
3-hydroxyadipate	162.05283	32.1	
adipoylcarnitine	289.15253	31.2	
3-hydroxy-3-methylglutarate	162.05284	31.0	x
2-isopropylmalate	176.06848	30.9	
N4-acetylcytidine	285.09608	30.7	x
3-hydroxyhippurate	195.05317	30.6	x
quinolinate	167.02187	30.2	x
methyl-4-hydroxybenzoate	152.04735	29.2	
p-cresol sulfate	188.01434	27.4	
pyridoxate	183.05317	26.1	
3-methoxycatechol sulfate	220.00417	25.4	
maltitol/lactitol/cellobiotol/palatinol	344.13187	25.0	
N-acetyl-1-methylhistidine	211.09551	24.9	x
ferulylglycine	251.07938	24.5	
4-vinylguaiacol sulfate	230.0249	23.4	
glucuronate	194.04266	23.2	
ferulic acid 4-sulfate	274.01473	23.1	
1-ribosyl-imidazoleacetate	258.08518	22.9	
thioproline	133.01974	22.7	
alpha-CEHC glucuronide	454.18391	22.3	x
2-hydroxyhippurate (salicylurate)	195.05317	22.3	
suberoylcarnitine	317.18383	22.3	

4-hydroxyphenylacetate	152.04736	22.3	
3-hydroxybutyroylglycine	161.06881	22.1	
N6-carbamoylthreonyladenosine	412.13427	21.6	
tigloylglycine	157.07388	21.5	
ethyl maltol sulfate	220.00417	20.8	
3,4-dihydroxyphenylacetate sulfate	247.99908	19.9	
hexanoylglutamine	244.14232	19.8	
indole-3-carboxylic acid	161.04769	19.6	
delta-CEHC glucuronide	426.1526	19.5	
ferulylglycine	251.07938	19.3	
suberate	174.08922	19.3	
5-hydroxyindole sulfate	213.00959	19.2	x
N-acetylpyrraline	296.13723	19.1	
2-methylbutyrylglycine	159.08955	18.9	
hydantoin-5-propionic acid	172.04842	18.7	
N1-methylinosine	282.09641	18.2	
indoleacetate	175.06334	18.1	x
N6-succinyladenosine	383.10772	17.8	
citramalate	148.03718	17.8	x
2,8-quinolinediol sulfate	241.0045	17.4	
3-methylglutarate/2-methylglutarate	146.05792	17.3	
N-acetylalliin	219.05654	17.3	
nonenedioate	186.08921	17.3	
pimeloylcarnitine/3-methyladipoylcarnitine	303.16818	17.1	
N-acetyltryptophan	246.10045	16.6	x
4-ethylphenylsulfate	202.02999	16.6	
phenol sulfate	173.99869	16.6	
benzoylcarnitine	265.1314	16.3	
isovalerylglycine	159.08955	15.6	x
S-adenosylhomocysteine (SAH)	384.1216	15.5	x
N-acetylmethionine sulfoxide	207.05652	15.4	
4-acetamidobutanoate	145.07388	15.4	x
3-methylcrotonylglycine	157.07388	14.8	
guanidinosuccinate	175.05932	14.6	x
3-hydroxycinnamate sulfate	244.00417	14.3	
vanillic alcohol sulfate	234.01982	14.2	
1-methylurate	182.04398	14.1	x
hydroxyasparagine	148.04839	13.9	
vanillylmandelate (VMA)	198.05284	13.8	x
4-vinylphenol sulfate	200.01434	13.6	x

N-(2-furoyl)glycine	169.03752	13.6	
lanthionine	208.05177	13.5	
methyl indole-3-acetate	189.07897	13.5	
3-hydroxybutyrylcarnitine	247.14196	13.3	
anthranilate	137.04767	13.0	
C-glycosyltryptophan	366.14271	12.9	
2-methylmalonylcarnitine	261.12123	12.9	
O-sulfo-L-tyrosine	261.03072	12.7	x
2-acetamidophenol sulfate	231.02015	12.5	
1-methyl-4-imidazoleacetate	140.05857	12.3	
2-butenoylglycine	143.05825	12.3	
argininosuccinate	290.12263	12.2	
1,7-dimethylurate	196.05965	12.0	x
phenylacetate	136.05244	11.4	x
imidazole propionate	140.05857	11.4	
5-methylthioribose	180.04562	11.3	
2-aminophenol sulfate	189.00959	11.3	
4-methylcatechol sulfate	204.00925	11.1	x
catechol glucuronide	286.06888	11.1	
2-hydroxyphenylacetate	152.04735	11.0	x
N-acetyl-isoputreanine	202.13173	10.9	
allantoic acid	176.05457	10.8	
N-acetylserine	147.05315	10.5	x
formiminoglutamate	174.06405	10.5	
fucitol	166.08413	10.4	
N-acetylaspartate (NAA)	175.04808	10.4	
N-acetylglucosaminylasparagine	335.13286	10.2	
5,6-dihydrouridine	246.0852	10.2	
sucrose	388.12171	10.0	x
N-acetylneuraminate	309.10597	9.8	x
o-cresol sulfate	188.01434	9.8	
2-methoxyresorcinol sulfate	220.00417	9.7	
3-methyl catechol sulfate	204.00925	9.6	
4-methylguaiacol sulfate	218.0249	9.4	
carboxyethyl-GABA	175.08445	9.4	
N-acetylcitrulline	217.10627	9.0	
carnosine	226.10658	8.9	
N-acetylphenylalanine	207.08955	8.8	
homocitrulline	189.11133	8.6	x
1,6-anhydroglucose	162.05283	8.5	

gamma-CEHC	264.13617	8.3	x
5-(galactosylhydroxy)-L-lysine	324.15326	8.3	
pimelate	160.07357	8.2	
indolelactate	205.0739	8.2	x
N-formylanthranilic acid	165.0426	8.1	
N-acetylmethionine	191.06163	7.9	
succinylcarnitine	261.12123	7.7	
3-hydroxyindolin-2-one sulfate	229.0045	7.7	
N-acetylthreonine	161.06882	7.6	
orotidine	288.05938	7.6	x
3-carboxy-4-methyl-5-pentyl-2-furanpropionate (3-Cmpfp)	268.13108	7.6	x
gulonate	196.05831	7.4	
2,3-dihydroxyisovalerate	134.05792	7.3	
5-hydroxymethyl-2-furoic acid	142.02662	7.3	x
mannitol/sorbitol	182.07905	7.2	
caffeic acid sulfate	259.99908	7.2	
ectoine	142.07422	7.1	
malonylcarnitine	247.10558	7.0	
N2,N2-dimethylguanosine	311.12296	7.0	x
phenyllactate (PLA)	166.063	6.9	
N-carbamoylalanine	132.05348	6.8	
xanthosine	284.07569	6.8	x
N-methylpipecolate	143.09462	6.8	
gamma-carboxyglutamate	191.04298	6.6	
pro-hydroxy-pro	228.111	6.6	
N-acetylasparagine	174.06405	6.5	
1H-indole-7-acetic acid	175.06334	6.5	
3-methoxycatechol sulfate	220.00417	6.4	
3-methyl catechol sulfate	204.00925	6.4	
pseudouridine	244.06955	6.4	x
phenylalanylglycine	222.10043	6.4	
1-methylhistidine	169.08514	6.4	x
azelate	188.10487	6.3	
glyco-beta-muricholate	465.30905	6.2	
hexanoylglycine	173.1052	6.2	
N2-acetyllysine	188.1161	6.1	
N-acetylhistidine	197.08005	6.1	x
heptenedioate	158.05792	5.9	
N-acetyltaurine	167.02524	5.6	
1,3,7-trimethylurate	210.0753	5.6	x

5-hydroxylysine	162.10043	5.5	
7-hydroxyindole sulfate	213.00959	5.5	
acisoga	184.12119	5.5	x
3-aminoisobutyrate	103.06332	5.4	x
hydroxy-CMPF	256.0947	5.4	
4-hydroxyglutamate	163.04806	5.4	
cytidine	243.08553	5.4	x
delta-CEHC	250.12052	5.3	
4-acetamidophenylglucuronide	327.09543	5.2	
sebacate (C10-DC)	202.12052	5.1	
methylmalonate (MMA)	118.02662	5.1	x
cytosine	111.04325	5.1	x
catechol sulfate	189.9936	5.1	
3-hydroxysebacate	218.11543	5.1	
2,3-dihydroxy-2-methylbutyrate	134.05792	5.0	
N-acetyl-2-aminooctanoate	201.1365	5.0	
arabitol/xylitol	152.06849	5.0	x
glutarylcarnitine	275.13688	4.9	x
3-methyladipate	160.07357	4.9	
hypoxanthine	136.0385	4.8	x
quinate	192.0634	4.8	
gentisate	154.02662	4.8	
dihydroferulic acid	196.07357	4.7	
tyramine O-sulfate	217.04089	4.7	
dodecanedioate	230.15182	4.6	
N1-methylguanosine	297.10733	4.6	
isobutyrylglycine	145.0739	4.5	x
propyl 4-hydroxybenzoate	180.07865	4.5	
N-formylmethionine	177.04598	4.5	
cysteine	121.01974	4.5	x
3-sialyllactose	633.21164	4.4	
5-acetylamino-6-amino-3-methyluracil	198.0753	4.4	x
N-acetylalanine	131.05825	4.4	x
N-acetyl-S-allyl-L-cysteine	203.06162	4.1	
trigonelline (N'-methylnicotinate)	137.04767	4.1	
1,2,3-benzenetriol sulfate	205.98852	4.0	
cortisol	362.20933	4.0	
saccharin	182.99902	3.9	x
aconitate	174.01645	3.9	x
N-acetylvaline	159.08955	3.9	x

N-acetylcarnosine	268.11715	3.8	
gamma-glutamylphenylalanine	294.12156	3.7	
N1-Methyl-2-pyridone-5-carboxamide	152.05859	3.7	x
myo-inositol	226.06888	3.7	x
alpha-ketoglutarate	146.02154	3.7	
kynurenine	208.08478	3.6	x
inosine	268.08078	3.6	
cysteine s-sulfate	200.97655	3.6	
gamma-glutamylvaline	246.12156	3.6	
creatinine	113.0589	3.6	x
cis-4-decenoylcarnitine	313.2253	3.5	
2-fucosyllactose	488.17413	3.5	
N-acetylglutamate	189.06373	3.5	
N6,N6,N6-trimethyllysine	188.15247	3.4	
N-acetyltyrosine	223.08447	3.4	
androstenediol (3beta,17beta) disulfate	225.06969	3.3	
N-acetylglucosamine/N-acetylgalactosamine	221.08991	3.3	
trans-4-hydroxyproline	131.05823	3.2	x
3-methylhistidine	169.08514	3.1	x
5-dodecenoylcarnitine	341.2566	3.1	
tiglylcarnitine	243.14705	3.1	x
N-acetylleucine	173.1052	3.1	
4-imidazoleacetate	126.04292	3.1	
alpha-ketoglutaramate	145.03752	3.1	
isobutyrylcarnitine	231.14705	3.0	
(N(1) + N(8))-acetylspermidine	187.16845	3.0	
prolylglycine	172.08478	2.8	
valerate	102.06809	2.8	
dimethylarginine (SDMA + ADMA)	202.14297	2.8	x
ribulonate/xylulonate	166.04775	2.7	
N-acetyl-beta-alanine	131.05825	2.7	
N-acetylglutamine	188.07972	2.7	
2-methylbutyrylcarnitine	245.1627	2.7	
3-methoxytyrosine	211.08445	2.7	
2-hydroxydecanoate	188.14125	2.7	
laurylcarnitine	343.27225	2.7	
undecanedioate	216.13617	2.7	
6-oxopiperidine-2-carboxylate	143.05825	2.6	
imidazole lactate	156.0535	2.6	
3-hydroxypyridine sulfate	174.99394	2.5	

lactose	388.12171	2.5	
5-hydroxyhexanoate	132.07865	2.5	
gamma-glutamylthreonine	248.10083	2.5	
urea	120.06472	2.4	x
X - 13726	380.0422	531.8	
X - 18935	229.00489	296.7	
X - 17353	508.21615	240.4	
X - 21821	244.08559	222.8	
glutamine conjugate of C8H12O2	268.14231	181.2	
X - 17685	234.02016	171.6	
glucuronide of C10H18O2	346.16279	171.6	
X - 17351	244.08519	164.1	
X - 12126	325.08119	157.0	
X - 17327	256.14314	140.7	
X - 12472	242.12664	125.7	
X - 11843	231.02027	117.7	
X - 13844	210.06444	114.0	
glucuronide of C10H18O2	346.16279	112.3	
X - 12117	203.12668	111.8	
X - 17365	283.05583	110.4	
X - 12739	242.12674	108.8	
X - 12812	209.06958	102.3	
X - 22508	325.08051	95.5	
X - 12261	259.01528	94.9	
X - 21840	348.17991	92.5	
X - 12830	373.12006	91.4	
X - 12263	276.03069	88.9	
glutamine conjugate of C8H12O2	268.14231	86.8	
X - 12199	262.09491	86.1	
X - 21831	364.17488	83.0	
X - 18838	339.09611	82.8	
X - 12718	325.08118	82.6	
X - 15503	194.06984	82.4	
X - 21803	213.01015	80.4	
X - 12013	243.02045	79.3	
X - 12262	259.01525	77.9	
X - 22509	474.15355	75.2	
glucuronide of C10H18O2	346.16279	72.7	
X - 12216	229.00472	71.9	
X - 12712	220.00452	71.1	

X - 24494	480.23498	69.6
X - 12714	304.07988	65.1
X - 12846	482.25077	64.2
X - 22475	285.12061	55.0
X - 12170	180.05387	53.1
X - 12849	332.18345	52.9
X - 13723	212.06889	52.8
X - 12733	340.07354	51.8
X - 17367	183.09018	50.6
X - 18240	195.05346	48.9
X - 13695	246.02	45.6
X - 22147	262.01501	45.4
X - 21792	198.08994	45.2
X - 12839	270.15884	44.9
glucuronide of C14H26O4	434.21522	44.4
X - 12410	275.04665	43.8
glutamine conjugate of C8H12O4	300.13215	43.7
X - 17354	474.15448	43.0
X - 07765	246.05567	42.5
X - 11838	277.00888	40.3
X - 21815	280.10691	40.3
X - 13729	242.9841	39.7
X - 21839	453.20166	39.4
X - 12701	320.07562	39.1
X - 18886	218.11575	39.0
glucuronide of C14H26O4	434.21522	38.8
X - 21816	114.10489	37.6
X - 16570	199.12161	36.5
X - 15486	270.15817	33.5
X - 21295	220.00433	31.8
X - 17346	254.09059	31.7
X - 18345	246.12176	29.6
X - 24334	416.19046	29.1
X - 24527	242.12637	28.8
X - 11850	227.0255	28.7
X - 12906	159.05345	28.0
X - 12636	258.15837	27.9
glucuronide of C12H22O4	406.18391	26.6
X - 24757	181.07376	26.2
X - 17686	260.03573	26.1

X - 23659	142.1104	25.4
X - 18887	329.15911	25.1
X - 13698	412.11624	22.9
X - 21796	139.06384	22.8
glucuronide of C8H16O2	320.14714	22.6
X - 12543	182.05827	22.3
X - 17361	332.18424	22.2
X - 21807	234.02082	22.2
X - 12015	217.09533	22.2
X - 23369	189.04225	21.8
X - 11979	250.07015	21.1
X - 24542	225.06393	20.8
X - 19141	416.20429	20.4
X - 24540	262.01504	20.0
X - 12411	196.04074	19.9
X - 23641	287.20933	18.8
glucuronide of C12H22O4	406.18391	18.7
X - 17359	542.27323	18.1
glucuronide of C10H18O2	346.16279	18.1
X - 12007	223.9993	17.9
glucuronide of C10H18O2	346.16279	17.8
X - 23652	170.06879	17.8
X - 12101	163.06657	17.2
glucuronide of C19H28O4	496.23085	16.8
X - 12738	232.00452	16.6
X - 13846	302.06421	16.6
X - 17676	168.05411	15.9
X - 12707	250.015	15.4
X - 24329	345.1169	14.7
X - 17340	540.25731	14.2
X - 17677	205.98883	14.2
glucuronide of C8H18O2	322.16279	14.1
X - 17328	307.17779	14.0
X - 13835	170.06905	13.8
X - 12206	255.98889	13.7
X - 11564	178.03029	13.5
X - 12462	147.03542	13.4
X - 14082	512.26276	13.3
X - 12565	253.15381	13.3
X - 23196	218.11526	13.3

X - 12283	244.08438	13.1
X - 24728	237.03032	13.1
X - 17348	248.09034	13.0
X - 12026	181.06036	13.0
X - 17692	371.10546	12.9
X - 24588	486.17444	12.8
X - 15492	542.27203	12.5
X - 24514	160.08446	12.2
glucuronide of piperine metabolite C17H21NO3	463.18425	12.2
X - 24452	204.14721	12.1
X - 17343	191.97323	11.7
X - 13553	264.03064	11.2
X - 13737	128.09508	11.2
X - 14838	140.05853	11.2
X - 11444	542.2725	10.9
X - 12729	229.00455	10.7
X - 12329	189.00981	10.6
X - 12407	205.98855	10.5
X - 17325	185.10534	10.5
X - 12753	219.05631	10.5
X - 17690	246.0559	10.4
X - 21312	244.04097	10.3
X - 21410	386.17693	10.1
X - 16124	204.00949	9.9
X - 24418	526.2766	9.5
glycine conjugate of C10H14O2	223.12085	9.5
X - 17299	228.14704	9.1
X - 24699	240.14707	8.8
X - 12100	220.08464	8.7
glucuronide of piperine metabolite C17H21NO3	463.18425	8.6
X - 24456	236.07935	8.5
glucuronide of piperine metabolite C17H21NO3	463.18425	8.2
X - 12221	205.00481	8.2
X - 12731	239.97667	7.8
X - 24809	216.14726	7.7
X - 21310	234.99187	7.6
X - 09789	154.02696	7.5
X - 16580	221.07185	7.4
X - 17146	376.18873	7.1
X - 11470	526.27786	7.0

X - 17357	542.27283	6.8
X - 17185	216.00928	6.7
X - 12844	540.25665	6.6
glycine conjugate of C10H12O2	221.1052	6.5
X - 23590	203.07907	6.5
X - 23776	145.11006	6.5
X - 21829	246.1475	5.9
X - 12111	143.05795	5.8
X - 22162	149.05099	5.8
X - 24328	162.08901	5.7
X - 12193	202.12131	5.7
X - 24766	114.07935	5.7
X - 24422	270.08481	5.6
X - 19561	253.09504	5.4
X - 23739	261.09557	5.4
glucuronide of C14H22O4	430.18391	5.2
X - 10458	232.13117	5.2
X - 17682	486.17435	5.1
X - 23587	131.09452	5.1
X - 11261	285.1935	4.9
X - 16397	247.08749	4.9
X - 12822	390.1001	4.7
X - 12230	218.02497	4.5
X - 13688	374.12211	4.4
X - 16087	444.16389	4.3
X - 23655	109.05292	4.3
X - 23649	271.06866	4.3
X - 24812	229.13132	4.2
X - 22102	203.11603	4.0
X - 23680	337.2247	4.0
X - 15497	237.10032	3.9
X - 12680	228.12196	3.9
X - 24974	219.0531	3.8
X - 13866	254.11559	3.8
X - 16964	175.06677	3.7
X - 21448	318.12201	3.7
X - 12212	230.02522	3.7
X - 15469	303.2043	3.6
X - 14056	191.02525	3.6
glucuronide of C12H20O3	388.17335	3.6

X - 23291	202.02987	3.5
X - 15666	220.08443	3.4
X - 24455	236.07942	3.3
X - 12104	270.08465	3.2
X - 23780	143.09442	3.2
X - 17438	246.14657	3.1
X - 21736	184.14645	3.0
X - 21851	510.2843	3.0
X - 12740	288.03058	2.9
X - 18889	203.11599	2.9
X - 13431	301.22486	2.9
X - 23644	145.07363	2.8
X - 24337	240.08585	2.8
X - 21319	166.09971	2.7
X - 24983	174.13645	2.6
X - 16944	142.09962	2.6
X - 13529	189.10013	2.6
X - 12726	234.02016	2.5
X - 24243	131.05817	2.5

HD/Control is ratio of mean plasma ultrafiltrate in hemodialysis and control subjects

Solutes named according to format “X-12345” are metabolites in the Metabolon database that have been identified in past studies but do not have known chemical structure.

CMPF: 3-carboxy-4-methyl-5-propyl-2-furanpropanoate

Supplementary Table 7: Variability of colon-derived solute peak areas

Colon-Derived Solute	Control Urine		Colectomy Urine		Control Ultrafiltrate		HD Ultrafiltrate	
	Detected #/17	CV	Detected #/12	CV	Detected #/14	CV	Detected #/14	CV
Identified as Uremic								
Phenylacetylglutamate	17	0.52	11	0.51	12	0.73	14	0.76
Cinnamoylglycine	17	0.79	8	1.34	9	0.73	14	0.98
p-Cresol glucuronide	17	1.32	1	-	14	1.35	14	1.31
Phenylacetylthreonine	16	0.80	1	-	9	1.25	14	1.26
Phenylacetylserine	14	0.64	2	1.14	5	0.99	14	0.91
Phenylacetylalanine	17	0.73	3	1.00	4	1.18	14	0.96
4-Acetylphenol sulfate	16	1.10	6	1.00	1	-	14	1.95
Phenylacetylmethionine	14	2.02	0	-	4	2.14	14	1.05
6-Hydroxyindole sulfate	17	0.36	4	1.66	14	0.50	14	0.43
Phenylacetylhistidine	17	0.60	3	0.98	6	0.66	14	0.81
3-(3-Hydroxyphenyl) propanoic acid sulfate	17	0.90	1	-	8	0.65	14	0.63
Trimethylamine N-oxide	17	1.41	12	1.14	13	0.87	14	0.54
Indoxyl sulfate	17	0.43	12	1.61	14	0.42	14	0.40
Phenylacetylglutamine	17	0.34	12	0.50	14	0.49	14	0.66
2-Oxindole-3-acetate	17	0.87	7	1.74	9	1.08	14	0.87
3-Hydroxyhippuric acid	17	0.89	8	1.31	14	0.94	14	0.58
p-Cresol sulfate	17	0.44	12	0.63	14	0.51	14	0.68
3-Methoxycatechol sulfate	17	1.60	12	1.56	13	0.94	14	0.59
Thioproline	13	0.79	2	0.93	4	1.42	14	0.60
Indoleacetic acid	17	0.82	12	0.98	14	0.26	14	1.04
2,8-Quinolinediol sulfate	15	0.92	3	1.23	1	-	12	1.38
4-ethylphenylsulfate	17	1.99	6	0.95	4	2.41	10	2.60
Phenol sulfate	17	0.97	12	0.61	14	0.87	14	0.55
Vanillic alcohol sulfate	17	1.46	10	1.02	12	1.65	13	1.13
2-Acetamidophenol sulfate	17	1.10	2	0.79	7	1.35	13	2.08
2-Aminophenol sulfate	17	1.25	9	1.84	14	1.23	14	1.02
4-Methylcatechol sulfate	17	1.38	8	1.46	14	1.94	14	0.78
Formylanthranilic acid	17	0.41	5	0.80	10	1.35	14	0.89
Azelaic acid	17	0.89	8	0.97	14	0.51	14	0.80

Pyrocatechol sulfate	17	0.76	11	1.55	14	0.77	14	0.50
Gentisic acid	17	1.70	8	1.07	9	1.47	14	0.67
1,2,3-Benzenetriol sulfate [a]	17	1.44	11	1.31	7	2.01	13	1.67
5-Hydroxyhexanoaic acid	11	0.95	1	-	14	0.26	14	0.67
Not Identified as Uremic								
CMPF	10	1.15	1	-	1	-	7	1.08
3-(3-Hydroxyphenyl) propanoic acid	10	1.15	0	-	9	0.89	14	0.92
5-Androstenediol disulfate	16	1.98	6	1.09	0	-	2	1.12
Picolinic acid	15	1.41	3	1.48	14	0.82	14	0.77
N-Methyltaurine	15	1.04	0	-	13	2.44	13	0.82
Fructose	14	1.08	5	1.59	14	0.46	14	0.50
1,2,3-Benzenetriol sulfate [b]	15	1.99	7	1.12	0	-	0	-
3-Hydroxyphenylacetatic acid	17	0.95	4	0.87	0	-	0	-
Indolepropionylglycine	17	0.79	1	-	0	-	0	-
N-Acetylhistamine	17	0.91	9	1.29	0	-	0	-
Piperidine	17	1.27	12	0.49	0	-	0	-
Pregnen-Diol disulfate	17	1.42	9	0.96	0	-	0	-
Triethanolamine	17	1.74	11	0.65	0	-	0	-

CV: Coefficient of variation calculated as the standard deviation of peak area / mean peak area. Coefficients of variation in urine samples were calculated using peak areas after filling missing values with half the minimum detected value each for solute in any urine sample and normalizing for body surface area. Coefficients of variation in plasma ultrafiltrates were calculated after filling missing values with half the minimum detected value for solute in any ultrafiltrate sample.

Compound names are those used by the Human Metabolomic Database (HDMB) except that CMPF is 3-carboxy-4-methyl-5-propyl-2-furanpropanoate.

[a] sulfate group on 2nd carbon and [b] sulfate group on 1st carbon in 1,2,3-Benzenetriol sulfate.

Supplementary Table 8: Urinary excretion of colon-derived solutes in colectomy patients with and without ileal pouches

Colon-Derived Solute	Neutral Mass	Urinary Excretion Rate Colectomy Without Pouch/ With Pouch	Ileal Pouch-Derived[c]	Urinary Excretion Rate Colectomy Without Pouch/ Control	Urinary Excretion Rate Colectomy with Pouch/ Control	Detected in Urine Colectomy Without Pouch (%)	Detected in Urine Colectomy with Pouch (%)	Detected in Urine Control (%)
p-Cresol glucuronide	284.0896	0.00		0.00	1.00	8	40	100
p-Cresol sulfate	188.01433	0.01	x	0.01	0.44	100	100	100
3-Hydroxyhippuric acid	195.05316	0.05	x	0.04	0.69	67	100	100
4-Methylcatechol sulfate	204.00924	0.08	x	0.04	0.46	67	100	100
2,8-Quinolinediol sulfate	241.00449	0.08	x	0.07	0.93	25	100	88
CMPF	240.09977	0.10		0.19	1.81	8	40	59
3-(3-Hydroxyphenyl) propanoic acid sulfate	246.01981	0.12		0.04	0.34	8	40	100
Phenylacetylalanine	207.08954	0.13		0.14	1.07	25	60	100
3-Hydroxyphenylacetatic acid	152.04735	0.14		0.11	0.77	33	60	100
Piperidine	85.08915	0.15	x	0.07	0.51	100	100	100
Triethanolamine	149.10519	0.15		0.14	0.97	92	100	100
4-Acetylphenol sulfate	216.00924	0.17	x	0.24	1.44	50	100	94
Phenylacetylhistidine	273.11134	0.19		0.11	0.56	25	60	100
6-Hydroxyindole sulfate	213.00958	0.20		0.08	0.42	33	40	100
Indoleacetic acid	175.06333	0.20	x	0.11	0.54	100	100	100
Indoxyl sulfate	213.00958	0.22		0.08	0.35	100	100	100
Azelaic acid	188.10486	0.24		0.22	0.93	67	80	100
N-Phenylacetylglutamatic acid	265.09502	0.25		0.18	0.73	92	100	100
Trimethylamine N-oxide	75.06841	0.25	x	0.14	0.55	100	100	100
Vanillic Alcohol Sulfate	234.01981	0.28		0.13	0.47	83	100	100
1,2,3-Benzenetriol Sulfate [a]	205.98851	0.28		0.11	0.38	58	80	88
Fructose	226.06887	0.28		0.20	0.70	42	100	82
N-Acetylhistamine	153.09021	0.31		0.18	0.58	75	100	100
Phenol sulfate	173.99868	0.32		0.23	0.71	100	100	100

5-Hydroxyhexanoaic acid	132.07864	0.33		0.12	0.37	8	40	65
Phenylacetylserine	223.08446	0.34		0.14	0.42	17	40	82
Picolinic acid	123.03203	0.35		0.16	0.46	25	100	88
Alpha-N-Phenylacetyl-L-Glutamine	264.11101	0.37		0.22	0.59	100	100	100
Formylanthranilic acid	165.04259	0.41		0.20	0.49	42	80	100
Pregnen-Diol disulfate	239.08475	0.41		0.21	0.51	75	100	100
Phenylacetylthreonine	237.10011	0.42		0.20	0.49	8	40	94
Thioproline	133.01975	0.43		0.25	0.57	17	80	76
5-Androstenediol disulfate	225.0691	0.44		0.15	0.34	50	100	94
3-Methoxycatechol sulfate	220.00416	0.49		0.19	0.38	100	100	100
Phenylacetylmethionine	267.09291	0.50		0.12	0.24	0	40	82
Cinnamoylglycine	205.07389	0.51		0.08	0.16	67	80	100
1,2,3-Benzenetriol sulfate [b]	205.98851	0.52		0.17	0.32	92	100	100
Gentisic Acid	154.02661	0.53		0.10	0.20	67	100	100
Pyrocatechol sulfate	189.99359	0.55		0.23	0.42	92	100	100
Indolepropionylglycine	246.10044	0.58		0.04	0.07	8	60	100
3-(3-Hydroxyphenyl) Propanoic Acid	166.06299	0.63		0.22	0.36	0	40	59
4-Ethylphenylsulfate	202.02998	0.71		0.01	0.02	50	80	100
2-Oxindole-3-Acetate	191.05824	0.73		0.13	0.17	58	100	100
2-Aminophenol sulfate	189.00958	0.86		0.12	0.14	75	100	100
N-Methyltaurine	139.03031	0.90		0.07	0.07	0	20	88
2-Acetamidophenol sulfate	231.02014	0.95		0.04	0.04	17	40	100
X - 12543	182.05826	0.02		0.01	0.70	83	100	100
X - 23997	222.06717	0.02		0.01	0.39	0	40	100
X - 17367	183.09017	0.08	x	0.06	0.75	33	100	94
X - 24757	181.07375	0.09	x	0.08	0.87	33	100	94
X - 15728	232.04068	0.10		0.08	0.86	0	40	82
X - 17438	246.14656	0.10	x	0.09	0.90	25	80	100
X - 24764	165.09982	0.10		0.16	1.52	75	80	100
X - 17325	185.10533	0.11	x	0.08	0.71	67	100	100
X - 12212	230.02521	0.13	x	0.15	1.19	25	80	88

X - 23657	143.09444	0.13		0.03	0.24	50	40	94
X - 13729	242.98409	0.14	x	0.07	0.49	25	80	100
X - 22508	325.0805	0.15		0.04	0.31	17	60	100
X - 12027	243.02026	0.15	x	0.04	0.26	8	80	65
X - 12718	325.08117	0.16		0.06	0.36	25	60	100
X - 24760	137.50476	0.17		0.08	0.49	17	20	82
X - 12740	288.03057	0.18	x	0.00	0.02	0	80	100
X - 21258	214.03044	0.19	x	0.22	1.20	17	80	71
X - 17686	260.03572	0.19		0.24	1.22	58	80	94
X - 12126	325.08118	0.24		0.07	0.30	8	40	94
X - 17673	149.98072	0.24		0.05	0.19	25	80	71
X - 13726	380.04219	0.24		0.17	0.70	0	20	47
X - 21310	234.99186	0.25		0.08	0.32	33	60	100
X - 17692	371.10545	0.25		0.24	0.96	25	40	71
X - 24272	275.0093	0.27		0.09	0.32	0	20	71
X - 12216	229.00471	0.27		0.05	0.19	50	100	100
X - 16071	145.05269	0.33		0.18	0.56	50	80	94
X - 12306	248.03574	0.41		0.10	0.25	17	40	100
X - 23583	115.06341	0.42		0.11	0.27	0	20	94
X - 21821	244.08558	0.43		0.05	0.12	8	60	100
X - 12283	244.08439	0.48		0.05	0.11	8	60	100
X - 17351	244.08518	0.50		0.05	0.11	8	60	100
X - 12013	243.02044	0.51		0.02	0.05	0	60	94
X - 24490	280.1058	0.62		0.07	0.12	100	100	100
X - 13866	254.11558	0.63		0.23	0.37	0	40	53
X - 12261	259.01527	0.66		0.10	0.15	0	20	59
X - 11843	231.02026	0.71		0.03	0.05	8	20	94
X - 12815	272.03571	0.75		0.03	0.04	0	20	53
X - 12830	373.12005	0.81		0.16	0.20	0	20	88
X - 17371	453.20085	0.81		0.11	0.13	0	20	76
X - 21845	357.12607	0.83		0.10	0.12	0	20	88
X - 21828	373.12114	0.85		0.10	0.12	0	20	82

X - 11640	378.0778	1.00	0.12	0.12	0	0	76
X - 17354	474.15447	1.00	0.05	0.05	0	0	94
X - 21839	453.20165	1.00	0.17	0.17	0	0	53
X - 22509	474.15354	1.00	0.08	0.08	0	0	94

Solutes named according to format "X-12345" are metabolites in the Metabolon database that have been identified in past studies but do not have known chemical structure. CMPF: 3-carboxy-4-methyl-5-propyl-2-furanpropanoate

[a], sulfate group on 2nd carbon and [b], sulfate group on 1st carbon in 1,2,3-Benzenetriol sulfate

[c], classified as ileal pouch derived if urinary excretion rates is lower in colectomy without ileal pouch than with ileal pouch by $q<0.05$ and ratio<0.25

Supplementary Table 9: Details of solutes identified as colon-derived uremic solutes in previous studies

The tables lists in two parts the 13 compounds which an extensive literature search (Supplementary Table 5) found were previously identified as colon-derived uremic solutes but were not identified as such in the current study. Five solutes had previously been identified as colon-derived solutes in human studies and were not identified as such in the current study. As shown in the table, four of these solutes were detected in 1 or fewer urine samples in the current study and classification as colon-derived was thus not possible. One solute, 2-methoxyphenol sulfate, was detected in the urine but the colectomy to control ratio of 0.33 missed our cutoff value of 0.25.

Previously Identified as Colon-Derived Uremic Solutes in Human Studies				
	Plasma Ultrafiltrate Hemodialysis/Control	Classified as Uremic Solute	Urine Colectomy/ Control	Classified as Colon-Derived Solute
5-Hydroxyindole	a	-	b	-
3-3-Sulfoxyphenyl propanoic acid	a	-	b	-
Indoxyl glucuronide	495.2	yes	b	-
Phenylacetic acid	11.4	yes	b	-
2-Methoxyphenol sulfate	45.2	yes	0.33	no

a, not detected in any ultrafiltrate samples so no ratio could be calculated and classification as a uremic solute was not possible.
b, detected in only 0 or 1 urine sample so no ratio could be calculated and classification as a colon-derived solute was not possible.

Eight solutes had previously been identified as colon-derived solutes in rodent studies and were not identified as such in the current study. As shown in the table, two of these eight solutes were detected in 1 or fewer urine samples in the present study and classification as colon-derived was thus not possible. The other six solutes did not meet our criteria for colon-derived solutes. Discrepancy in colon-derived solutes in human and rodent studies have been noted in past studies. For instance, hippurate has been categorized as colon-derived in previous rodent studies (supplementary table 5), but not in past human studies (13). This presumably reflect production of hippurate by mammalian cells including glycine conjugation of ingested benzoic acid in the liver. We presume some other solute which have been identified as colon-derived in rodent studies may also be produced by human cells. In other cases, difference in identification may result from statistical variation and/or errors in measurement in either our study or the prior studies.

Supplementary Table 9 (continued)

Previously Identified as Colon-Derived Uremic Solutes in Rodent Studies				
	Plasma Ultrafiltrate Hemodialysis/Control	Classified as Uremic Solute	Urine Colectomy/ Control	Classified as Colon-Derived Solute
2-Hydroxyvaleric acid	a	-	b	-
Cholate	a	-	b	-
Phenylacetylglycine	33.8	yes	0.32	no
Hippurate	36.7	yes	0.54	no
4-Guanidinobutyric acid	1.1	no	0.84	no
Succinate	1.2	no	0.72	no
Glutarate	2.1	no	0.49	no
Dimethylglycine	2.3	No	0.60	no

a, not detected in any ultrafiltrate samples so no ratio could be calculated and classification as a uremic solute was not possible. b, detected in only 0 or 1 urine sample so no ratio could be calculated and classification as a colon-derived solute was not possible.

Supplementary Table 10: Colon-derived solutes identified as Organic Acid Transporter (OAT) substrates

First Author	Deguchi (16)		Wu (17)		Hseuh (18)	
Year	2004		2017		2016	
PMID	14675047		28694431		27467266	
Model	Cell Culture		OAT1 Knockout Mice	OAT3 Knockout Mice	Cell Culture	
	OAT1 Substrate	OAT3 Substrate	OAT1 Substrate	OAT3 Substrate	OAT1 Inhibitor	OAT3 Inhibitor
2-aminophenol sulfate				x		
2-oxindole-3-acetate				x		
CMPF	x	x		x	x	x
Indoxyl sulfate	x	x	x	x	x	
Catechol sulfate				x		
Indoleacetic acid	x			x		
p-Cresol sulfate				x	x	x
Phenol sulfate			x	x		
TMAO				x		

CMPF: 3-carboxy-4-methyl-5-propyl-2-furanpropanoate; TMAO Trimethylamine N-oxide

Supplementary Table 11: Sensitivity analysis showing the number of colon-derived uremic solutes with varying cutoff values for defining colon-derived solutes and uremic solutes.

		Threshold for Colon-Derived Solutes		
		2-Fold	4-Fold	8-Fold
Threshold for Uremic Solutes	2-Fold	79/124	61/91	37/57
	2.4-Fold	78/124	60/91	37/57
	4-Fold	72/124	55/91	34/57
	8-Fold	63/124	50/91	32/57

Figures represent the number of colon-derived uremic solutes / number of colon-derived solutes for each set of criteria. Solutes were categorized as colon-derived if urinary excretion rate in colectomy / normal subjects met listed threshold and false detection rate $q < 0.05$ for difference between groups. Solutes were categorized as uremic if plasma ultrafiltrate in hemodialysis / normal subjects met listed threshold and false detection rate $q < 0.05$ for difference between groups.

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