Kidney Medicine

Metabolomics to Identify Unclassified Uremic Toxins: A Comprehensive Literature Review

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A comprehensive review of known uremic retention molecules goes back to more than 10 years ago and did not consider metabolomic analyses. The present analysis searches for as of yet unclassified solutes retained in chronic kidney disease (CKD) by analyzing metabolites associated with relevant outcomes of CKD. This untargeted metabolomics-based approach is compared with a conventional targeted literature search. For the selected molecules, the literature was screened for arguments regarding toxic (harmful), beneficial, or neutral effects in experimental or clinical studies. Findings were independently cross-checked. In total, 103 molecules were selected. No literature on any effect was found for 55 substances, 3 molecules had no significant effect, and 13 others showed beneficial effects. For the remaining 32 compounds, we found at least one report of a toxic effect. Whereas 62.5% of the compounds with at least one study on a toxic effect was retrieved via the bottom-up approach, 69.2% of the substances originating from metabolomics-based approaches showed a beneficial effect. Our results suggest that untargeted metabolomics offer a more balanced view of uremic retention than the targeted approaches, with higher chances of revealing the beneficial potential of some of the metabolites.

Complete author and article information provided before references.

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Chronic kidney disease (CKD) is a major but much neglected public health problem. CKD affects 15%-20% of adults,[1] costs more to societies than other common chronic diseases[2] and is the fastest rising cause of death worldwide.[3] Life expectancy is reduced by >50% from the early stages on.[1] Cardiovascular disease,[1,4,5] cancer,[6] and infection[7] are the main causes of death. This multifactorial increase in mortality is related to a progressive deterioration of organ functions and cell metabolism, with a central role attributed to the retention of a broad array of solutes that normally are excreted by the kidneys into the urine.[8-10] Increasing interest in and knowledge about the biological, biochemical, and clinical impact of those solutes unraveled a complex network of alterations that accelerate as kidney disease progresses, giving rise to the uremic syndrome.[11]

The European Uremic Toxin Workgroup (EUTox)[12] issued 2 encyclopedic lists of uremic retention solutes, containing in total 146 different solutes or groups of solutes.[8,9] However, these 2 initial publications reported retained solutes without specifically focusing on their toxic (harmful) impact. A subsequent review based on the 90 compounds of the first overview,[9] showed a toxic biological, biochemical or clinical impact for 66 substances or groups of substances.[10]

Most solutes reported in these publications had been identified by classic targeted analytical methods (chemical analysis, electrophoresis, enzyme-linked immunosorbent assay, and spectrometry) implying the analysis of predefined solutes, of which mostly an experimental effect and/or correlation with clinical outcomes had been demonstrated via separate independent studies.

In contrast, metabolomics follows a reverse approach and explores in an untargeted way a broad array of solutes to define which correlate to predefined outcomes or effects.[13] A recent explosion of studies applying high-throughput and high-resolution metabolomic methodologies in CKD allowed the untargeted detection of solutes, that were correlated to relevant negative outcomes, such as overall mortality, cardiovascular events, progression of kidney insufficiency or cognitive dysfunction.[14] However, it usually remains unclear whether these compounds induce pathophysiological effects, although this would fully qualify them as uremic toxins.[14]

The present hypothesis-generating review presents an analysis of the results of several top-down metabolomic publications with significant relevance to the outcomes of patients with CKD and of one bottom-up review of uremic retention solutes, aiming at identifying whether those solutes have been independently linked to pathophysiologic effects or negative clinical outcomes. Based on the retrieved information, a number of suggestions for future metabolomic analyses are formulated to optimize the understanding of the mechanisms that underly the uremic syndrome.

METHODS

Literature Search

A literature search was conducted in PubMed with the search terms: CKD, chronic kidney disease, uremic or uremia on one hand, and metabolomic on the other. Among the identified references, studies were selected that

- In total, 103 molecules were selected for analysis of their clinical or experimental impact
- No literature on any effect was found for 55 molecules
- 3 molecules had no significant effect
- 13 molecules showed a beneficial effect
- For the remaining 32 compounds, at least one report on a toxic effect was found, but for several of these we found more reports on benefits than reports on harm
- Three of the 4 solutes with the highest number of reports on a toxic effect were the result of post-translational modifications (malondialdehyde, 4-OH hexanal, carboxymethyllysine)
- The targeted bottom-up approach yielded proportionally more toxic molecules than the untargeted top-down approach
- Most solutes with a beneficial effect showed an increased concentration in CKD
- Interesting molecules that deserve more study are choline, anthranilic acid and pentosidine

searched for solutes that were correlated with major outcomes of CKD: overall mortality, cardiovascular morbidity or mortality, progression of CKD and/or cognitive dysfunction, or metabolite generation by the gut microbiome.[14] The latter endpoint has been selected because of the key role of the intestinal metabolism in the generation of uremic toxins but also of beneficial metabolites. This selection process identified 11 studies published between 2011 and 2020 based on their citations and/or the impact of the journal in which they were published.[15-25]

From these studies, substances that showed a significant association with outcomes were identified. Only plasma or serum concentrations were considered. For studies assessing both an exploration and an independent validation cohort,[16-18,23,24] essentially solutes which maintained their significance in the validation arm were taken into account.[26] We also included uremic solutes already identified in the previous European Uremic Toxins Work Group (EUTox) reviews,[8,9] but excluded those that had previously been characterized for their biological and/or clinical impact in a review[10] published in 2018. This approach aimed at searching for solute toxicity and allowed comparing the yield of the top-down approach based on untargeted metabolomics with the classic bottom-up methodology of the previous EUTox searches.[8,9]

Each of the selected substances was submitted to a literature search via PubMed for evidence of experimental or clinical impact (either beneficial, neutral, or toxic) on outcomes relevant to patients with CKD, including also biological, biochemical or molecular changes related to such outcomes (e.g., inflammation, insulin resistance, etc.) (Table 1). Only studies with the name of the targeted substance in the title were considered. Because several of the substances of interest could be reported either as a salt or as an acid (e.g., butyrate vs. butyric acid), for all such substances both terms were searched.

Table 1. Most Important Considered Effects[a]

Cardiovascular disease
o Cardiovascular events
o Intervention for cardiovascular events
o Hypertension
o Heart failure
o Arrythmia
Inflammation
Metabolic changes
o Liver dysfunction
o Enzymatic dysfunction
o Organic transport pump dysfunction
Neurologic disturbances
o Cognitive dysfunction
o Polyneuropathy
o Itching
o Dementia
o Parkinsonism
Insulin resistance
Bone disorder
o Mineral and bone disorder
o Bone fractures
o Osteoporosis
o Osteopenia
Fibrosis
o Kidney fibrosis
▪ Progression of kidney dysfunction
o Cardiac fibrosis
Coagulation disturbances
o Thrombogenicity
o Bleeding tendency
Hematologic changes
o Anemia
o Leukocytopenia
o Thrombocytopenia
Endocrinologic changes
Muscular changes
o Muscular dystrophy
o Muscular dysfunction
o Sarcopenia
Gastro-intestinal changes
o Gastric ulcer
o Colon ulcer
o Motility changes
o Loss of appetite
Carcinogenicity
o Increased proliferation of cancer cells
Genetic modifications
Susceptibility to infection

[a]This list contains examples of the most important effects that were considered and is not exhaustive.

In the exceptional case that the yield exceeded 50 publications, the search was refined by the terms uremic, uremia, CKD, toxin or toxicity as additional keywords. Only original publications and no letters, abstracts, reviews or editorials were considered. The content of the full text of the paper were screened in detail to verify whether the findings were relevant or not, and studies considered irrelevant were not further analyzed. The analysis was restricted to the years 2017-2020. All relevant publications were retrieved together at once early in 2021, before screening for validity was started.

The initial literature search was undertaken by one of the authors (RV) after which the list of considered substances was distributed randomly among the remaining

authors for further analysis and confirmation. This approach yielded, after careful reconsideration, several additional references and changes in interpretation. The findings of this analysis and subsequent corrections were all summarized in the supplementary file (Item S1: Supplemental Data – Summary Search Results).

Toxicity Analysis

For toxicity analysis, we only considered substances with at least one study reporting a toxic impact or negative outcome. We did not further analyze solutes with no reported toxicity or with reported benefit, but noted the identity of these substances and the references of the specific reports indicating these effects (supplemental data – beneficial effects and comments).

All reported effects (either toxic or not toxic) per affected organ or pathophysiological system for each considered study were noted in a point-by-point overview table. The table indicated per substance, study and organ or system, the outcome ("-" for an indication of toxicity, "+" for a neutral or beneficial result), and the design of the study ("E" for experimental and "C" for clinical).

This approach allowed an aggregated overview of all impacts reported for those molecules with at least one clinical or experimental report related to a toxic effect.

RESULTS

Eleven metabolomic studies were selected for further analysis (Table 2).[15-25] Nine of these studies were performed in patients with CKD or hemodialysis (81.8%), 1 study evaluated the general population,[22] and 1 analyzed CKD in mice.[15-18,20-25]

Based on these studies and a previous review of uremic retention solutes by Duranton et al,[8] a total of 103 substances of interest were identified (Table 3). Thirty-nine of these substances (37.9%) were recovered from the publication by Duranton et al,[8] whereas all other substances (n = 64, 62.1%) originated from the 11 untargeted metabolomic studies. Although the bottom-up approach[10] only reported substances with increased blood/plasma levels, metabolomics led to the identification of 23 (36%) solutes with increased levels, 2 (3%) with unchanged, another 2 (3%) with decreased levels, and 37 (60%) for which the concentration or fold changes were not available (either not reported or not available by design) (Table 3).

No reports on toxic effects were found for 71 (68.9%) of these substances, which corresponds with no report at all for 55 (53.4%), no significant effect in either direction for 3 (2.9%), and only beneficial effects for 13 (12.1%) solutes. These solutes are listed and commented in the supplementary file (**Item S2: Beneficial Effects and Comments**).

For the remaining 32 solutes, at least one report pointing to a toxic effect was identified and all reports found about these solutes were subjected to an in-depth analysis. In total, 315 publications were screened for evidence of biological or clinical impact of those substances of which 215 were considered to contain relevant information. An overview of those findings can also be found in the supplemental data (overview search results) and in a comprehensive Table S1 (supplemental data - summary table of studies). Of these 32 solutes, 62.5% came out of the review article by Duranton et al.[8] On the other hand, of the 13 solutes for which only studies reporting benefits were identified, only 4 (30.8%) were reported by Duranton et al,[8] and 9 (69.2%) were retrieved from metabolomic studies. In addition, also of the 55 compounds for which no reports could be found, only 14 (25.4%) originated from Duranton et al.[8]

Figure S1A shows that experimental data were found for almost all solutes subjected to this in-depth analysis and that there is an almost equal distribution of reports showing no toxicity per organ system for the majority of studies (neutral or beneficial effect, illustrated by a blue shade) and reports showing a pathophysiologic effect per organ system for the majority of studies (illustrated by a red shade). An orange shade was used in case an equal number of studies reported toxicity versus no toxicity. Most studies assessed the impact on the cardiovascular system, metabolism and inflammation and only a few evaluated muscular wasting, thrombogenicity and susceptibility to infection (Figure S1).

Regarding the specific solutes, on one hand results suggesting toxicity in more than 75% of studies were found for 4-OH nonenal, cholate, cysteine, glutarate, intercellular adhesion molecule, malondialdehyde, and propionic acid (Table 4). On the other hand, more than 75% of studies suggesting no toxicity were found for α_1-acid microglobulin, butyrate, calcitonin, neopterin, noradrenaline, and thiocyanate. Results for choline, citric acid, insulin-like growth factor (IGF), and vascular-endothelial growth factor (VEGF) pointed almost equally to toxicity and no toxicity.

Figure S1B visualizes the results of the clinical studies. Compared with Figure S1A, substantially less studies could be retrieved, illustrating the relative scarcity of data on the clinical associations of uremic retention solutes as compared with experimental data. Also, the number of substances for which data could be found was lower. Most clinical studies were observational and pointed to an association with a toxic effect (Table 4), in contrast to the more balanced distribution for the experimental data (Figure S1A). Again, the largest number of studies were devoted to cardiovascular disease and inflammation, but, compared with the experimental studies, relatively more attention was given to neurologic disorders, insulin resistance, and bone disease. Only one study was retrieved on thrombogenicity, hematologic and endocrinologic changes, muscular wasting and gastrointestinal disease, whereas no study assessed carcinogenicity, genetic modifications and susceptibility to infection (Figure S1B).

Table 2. Metabolomic Studies Screened For Retrieval Of Potentially Relevant Molecules

First Author	Journal	Year	Study Population (Number)	Sample	Endpoint / Studied Parameter	Validation (Number)	Statistical Approaches	Ref
Aronov et al[15]	*J Am Soc Nephrol*	2011	HD patients +/- colectomy and controls (n = 6/n = 9 and n = 7-10)	Plasma	Intestinal generation	No	- Direct subgroup comparison - *P* values adjusted for multiple testing	15
Kalim et al[16]	*J Am Heart Assoc*	2013	Incident HD patients and controls (n = 100 and n = 100)	Plasma	Cardiovascular mortality	Yes (n = 100 and n = 200)	- Nested case-control study - Correction for multiple testing and multivariable adjustment	16
Kurella-Tamura et al[17]	*J Am Soc Nephrol*	2016	HD patients (n = 141)	Plasma	Cognitive dysfunction	Yes (n = 180)	- Correction for multiple testing and adjustment for demographic and clinical parameters	17
Luo et al[18]	*Clin J Am Soc Nephrol*	2019	Patients with CKD (n = 962)	Serum	Proteinuria Kidney failure	Yes (n = 620)	- Cross-sectional association study - Correction for multiple testing and adjustment for key clinical parameters	18
Mishima et al[19]	*Kidney Int*	2017	GF+SPF control and CKD mice (n = 4 and n = 6)	Plasma	Intestinal generation	No	- Direct subgroup comparison - Correction for multiple testing	19
Niewczas et al[21]	*Kidney Int*	2014	T2DM + patients with CKD (n = 80)	Plasma	Kidney failure	No	- Nested case-control study - Adjustment for multiple comparisons and for albumin excretion, eGFR, and HbA1c levels	21
Niewczas et al[20]	*Diabetes Care*	2017	Patients with T1DM + CKD (n = 158)	Serum	Kidney failure	No	- Prospective cohort study - Adjustment for relevant clinical covariates	20
Rhee et al[22]	*J Am Soc Nephrol*	2013	General population (n = 1434)	Plasma	CKD	No	- Nested case-control study - Adjustment for multiple comparison and for eGFR, age, sex, diabetes, hypertension, proteinuria.	22
Sekula et al[23]	*J Am Soc Nephrol*	2016	Non-CKD and CKD (n ≤ 1735)	Serum	Kidney function	Yes (n = 1164)	- Cross-sectional association study - Multivariable adjustment	23
Sharma et al[24]	*J Am Soc Nephrol*	2013	Healthy controls Diabetes patients +/- CKD (n=16 and n=16)	Plasma	CKD, Mitochondrial dysfunction	No[a]	- Adjustment for multiple comparison and multivariable adjusted Cox-model analysis	24
Titan et al[25]	*Plos One*	2019	Patients with CKD (n=454)	Serum	Mortality, Kidney failure	No	- Direct subgroup comparison - Adjustment for multiple comparison and multivariable adjusted Cox models	25

[a]In the study by Sharma et al only urinary, not plasma, metabolome data were validated in an independent cohort. Abbreviations: T2DM: diabetes mellitus type 2; T1DM: diabetes mellitus type 1; CKD: chronic kidney disease; GF: Germ free; SPF: Specific Pathogen-free; HD: hemodialysis; eGFR: estimated glomerular filtration rate; HbA1c: hemoglobin A1c.

Table 3. Retained Molecules, the Publication of Origin, and Their Effect

Solute	First Author	Effect[a]	Level Change[b]
2-Ethyl-3-OH propionate	Sharma et al[24]		↑
2-Heptenal	Duranton et al[8]		↑
2-Hexenal	Duranton et al[8]		↑
2-hydroxybutyrate	Niewczas et al[21]	≥1 Toxic	NM
2-Hydroxyisocaproate	Niewczas et al[21]	Beneficial	NM
2-Hydroxyisovalerate	Niewczas et al[21]		NM
2-Hydroxypentanoate	Mishima et al[19]		↑
2-Nonenal	Duranton et al[8]		↑
2-Octenal	Duranton et al[8]		↑[c]
2-O-glycerol-a-D-pyranoside	Titan et al[25]		NM
2-Oxoisocaproate	Niewczas et al[21]		NM
2-Oxoisoleucine	Niewczas et al[21]		NM
3-Dehydrocarnitine	Niewczas et al[21]		NM
3-Hydroxy isobutyrate	Sharma et al[24]		↓
3-Hydroxy isovalerate	Sharma et al[24]		↑
3-Hydroxy propionate	Sharma et al[24]		=
2-Methyl acetoacetate	Sharma et al[24]		NM
3-Methyl adipic acid	Sharma et al[24]		NM
3-Methyl crotonyl glycine	Sharma et al[24]		NM
4-Acetamitolbutanoate	Niewczas et al[21]		NM
4-Decenal	Duranton et al[8]		↑
4-Hydroxyhippurate	Sharma et al[24]		NM
4-Hydroxyphenylacetate	Kurella-Tamura et al[17]		NM
4-Hydroxyproline	Sharma et al[24]	≥1 Toxic	NM
4-OH-Hexenal	Duranton et al[8]	≥1 Toxic	↑
4-OH-Nonenal	Duranton et al[8]	≥1 Toxic	↑
4-OH-Octenal	Duranton et al[8]		↑
4-Pyridone-3-carboxamide-1-β-D-ribonucleoside	Duranton et al[8]		↑
5-Hydroxyindole acetic acid	Rhee et al[22]		NM
6-Acetamidobuturoate	Luo et al[18]		NM
8-Hydroxy-2'-deoxyguanosine	Duranton et al[8]	≥1 Toxic	↑
Aconitate	Rhee et al[22]/Sharma et al[24]	Beneficial	NM/↑
Anthranilic acid	Duranton et al[8]	≥1 Toxic	↑
Arabitol	Niewczas et al[21]		NM
Argininic acid	Duranton et al[8]		↑
Butyrate	Mishima et al[19]	≥1 Toxic	NM
Calcitonin	Duranton et al[8]	≥1 Toxic	↑[d]
Carboxymethyllysine	Duranton et al[8]	≥1 Toxic	↑
C-glycosyltryptophan	Niewczas et al[21]		NM
Cholate	Mishima et al[19]	≥1 Toxic	↑
Choline	Rhee et al[22]	≥1 Toxic	NM
Cinnamoylglycine	Aronov et al[15]		↑
Citric acid	Sharma et al[24]	≥1 Toxic	↑
Citrulline	Rhee et al[22]	Beneficial	NM
C-Mannosyl-tryptophan	Sekula et al[23]		↑
C-Glycosyltryptophan	Niewczas et al[20]		↑
Cysteine	Duranton et al[8]	≥1 Toxic	↑
Decanal	Duranton et al[8]		↑
Dihydroxyphenylalanine	Duranton et al[8]		↑
Dimethylglycine	Mishima et al[19]	Beneficial	↑
Erythritol	Niewczas et al[21]	≥1 Toxic	NM
Erythronate	Niewczas et al[21]		NM
Ethylamine	Duranton et al[8]	Beneficial	↑
Gamma-aminobutyric acid	Rhee et al[22]	Beneficial	NM
Glutarate	Mishima et al[19]	≥1 Toxic	↑

(Continued)

Table 3 (Cont'd). Retained Molecules, the Publication of Origin, and Their Effect

Solute	First Author	Effect[a]	Level Change[b]
Glutaroyl carnitine	Niewczas et al[21]		NM
Glutathione	Duranton et al[8]	Beneficial	↑
Glycolic acid	Sharma et al[24]		=
Guanidino butyrate	Mishima et al[19]		↑
Heptanal	Duranton et al[8]		↑[e]
Hexanal	Duranton et al[8]	≥1 Toxic	↑
Homovanillic acid	Sharma et al[24]	Beneficial	NM
Hydroxyindole	Aronov et al[15]	≥1 Toxic	↑
Intercellular adhesion molecule-1	Duranton et al[8]	≥1 Toxic	↑
Insulin-like growth factor-1	Duranton et al[8]	≥1 Toxic	↑
Isocitrate	Rhee et al[22]	Beneficial	NM
Lactose	Titan et al[25]		NM
Malondialdehyde	Duranton et al[8]	≥1 Toxic	↑
Myoglobin	Duranton et al[8]	≥1 Toxic	↑
N2,N2-Dimethylguanosine	Niewczas et al[21]		NM
N2,N5-Diacetylornithine	Luo et al[18]		NM
N4-Acetylcytidine	Niewczas et al[21]	≥1 Toxic	NM
N6-Carbamoylthreonyladenosine	Luo et al[18]		NM
N-Acetylalanine	Niewczas et al[20]		↑
N-Acetylcarnosine	Niewczas et al[20]		↑
N6-Carbamoylthreonyladenosine	Niewczas et al[20]		↑
N-Acetylserine	Niewczas et al[20]		↑
N-Acetylthreonine	Niewczas et al[20]		↑
Neopterin	Duranton et al[8]	≥1 Toxic	↑
Nicotinamide	Duranton et al[8]	Beneficial	↑
N-Methyl-2-pyridone-5-carboxamide	Duranton et al[8]		↑
N-Methyl-4-pyridone-5-carboxamide	Duranton et al[8]		↑
Nonanal	Duranton et al[8]		↑
Noradrenaline	Duranton et al[8]	≥1 Toxic	↑
Oleoylcarnitine	Kalim et al[16]		↑
Osteocalcin	Duranton et al[8]	≥1 Toxic	↑
O-Sulfotyrosine	Niewczas et al[20]		NM
Pentosidine	Duranton et al[8]	≥1 Toxic	↑
Phenaceturate	Mishima et al[19]		↑
Phenylglucuronide	Aronov et al[15]		↑
Propionate	Mishima et al[19]	≥1 Toxic	↓
Salicylic acid	Aronov et al[15]	Beneficial	↑
Succinate	Mishima et al[19]		↑
Thiocyanate	Duranton et al[8]	≥1 Toxic	↑
Threonine	Niewczas et al[21]	Beneficial	NM
Tiglylglycine	Sharma et al[24]		NM
Uracil	Sharma et al[24]	≥1 Toxic	NM
Vascular-endothelial growth factor	Duranton et al[8]	≥1 Toxic	↑
Xanthoine	Rhee et al[22]		NM
α-1-Acid-glycoprotein	Duranton et al[8]	≥1 Toxic	↑
α-1-Microglobulin	Duranton et al[8]	≥1 Toxic	↑
α-Keto-δ-guanidinovaleric acid	Duranton et al[8]		↑
β-Trace protein	Duranton et al[8]	Beneficial	↑

Note: Italics indicate solutes for which only one or more benefits were found (see SUPPLEMENTAL MATERIAL, OVERVIEW SEARCH RESULTS); underscore includes solutes with at least one report pointing to a toxic impact, but for which the benefits overruled the toxicity (Table 4).
[a]Effect: Beneficial, only references with beneficial effects were found; ≥1 toxic, at least one reference with a toxic effect was found, which does not exclude also references reporting beneficial effects for the same molecule.
[b]Level change: concentration change in CKD versus normal or better kidney function; ↑: increase; ↓: decrease; =: no significant differences; NM: not mentioned. Methodology used in CKD and in controls not necessarily the same for data reported in Duranton et al.[8]
[c]CKD value only 24.5% above normal.
[d]CKD value only 9% above maximum normal value.
[e]CKD value only 19.6% above normal. All other CKD values exceeded normal values by more than 25%.

Table 4. Evidence Data on Clinical and Experimental Impact per Individual Molecule

	Clinical[a]	Experimental[a]	Source	Notes
2-OH Butyrate	1/0	NA	Niewczas et al[21]	
4-OH-Hexenal	0/1	0/3	Duranton et al[8]	
4-OH-Nonenal	NA	1/8	Duranton et al[8]	
4-OH Proline	0/1	2/0	Sharma et al[24]	
8-OH-2-Deoxyguanosine	0/1	NA	Duranton et al[8]	
α1 Acid glycoprotein	NA	4/2	Duranton et al[8]	
α1 Acid microglobulin	NA	11/1	Duranton et al[8]	
Anthranilic acid	0/1	NA	Duranton et al[8]	- Key position in tryptophan metabolism, in between the toxins kynurenic acid and quinolinic acid - Extended search outside the preset timeframe disclosed more elements in favor of a biologic and clinical impact
Butyrate	1/0	21/3	Mishima et al[19]	
Calcitonin	NA	7/2	Duranton et al[8]	
Carboxymethyllysine	0/4	1/2	Duranton et al[8]	
Cholate	NA	0/9	Mishima et al[19]	
Choline	NA	5/3	Rhee et al[22]	- Precursor of the vasculotoxin TMAO - Neuroprotective at low doses, vasculotoxic at higher doses - Needs more extensive study
Citric acid	3/0	3/5	Sharma et al[24]	- Toxic at extremely high concentrations - At lower concentrations successfully used for local anticoagulation in dialysis - In dialysis anti-inflammatory
Cysteine	NA	0/4	Duranton et al[8]	
Erythritol	1/0	2/1	Niewczas et al[21]	
Glutarate	NA	0/5	Mishima et al[19]	
Hexanal	NA	0/2		
Hydroxyindole	NA	0/1	Aronov et al[15]	
Insulin-like growth factor	NA	8/5	Duranton et al[8]	- Vasculotoxic and oncogenic - Antioxidant and neuroprotective
Intercellular adhesion molecule-1	0/3	0/5	Duranton et al[8]	
Malondialdehyde	0/11	0/6	Duranton et al[8]	
Myoglobin	NA	0/3	Duranton et al[8]	- In vitro toxic for tubular cells, conform with clinical observations in crush syndrome and rhabdomyolysis - However, functional conditions in CKD differ from those in rhabdomyolysis
N4-acetylcytidine	NA	0/1	Niewczas et al[21]	
Neopterin	0/4	4/0	Duranton et al[8]	- Clinical marker of negative CV outcomes - In experimental studies, vasculoprotective
Noradrenaline	1/2	7/1	Duranton et al[8]	- Toxic for cardiovascular system - Neuroprotective
Osteocalcin	5/1	4/1	Duranton et al[8]	
Pentosidine	2/9	NA	Duranton et al[8]	- Within the present timeframe (2017-2020) only clinical data - Outside that timeframe a few studies showed an experimental impact - More experimental data needed
Propionic acid	1/0	2/12	Mishima et al[19]	- Toxic at concentrations that are too high for CKD - In CKD, concentrations are lower than with normal kidney function
Thiocyanate	1/2	4/1	Duranton et al[8]	
Uracil	NA	1/3	Sharma et al[24]	- Oncogenic only at very high concentrations
Vascular-endothelial growth factor	NA	10/8	Duranton et al[8]	- Vasculotoxic, profibrotic and oncogenic - Tissue protection and regeneration

Abbreviations: NA, no studies available; TMAO, trimethylamine-N-oxide.
[a]Number of studies found with either a beneficial or neutral impact (before the slash) or a toxic impact (after the slash).

A uniform trend for results suggesting an association with a pathophysiologic effect in at least 75% of studies was found for carboxymethyllysine, malondialdehyde, neopterin, and pentosidine. Apart from osteocalcin, there were no compounds with more than 75% of studies pointing to an absent toxic effect. For propionic acid, the experimental and clinical data were asymmetric, with the majority of experimental studies pointing to a toxic effect, whereas the one retained clinical study showed no toxic effect. For neopterin, all experimental studies showed a benefit and all clinical data suggested an association with toxicity. Also, for 4-OH proline, citric acid, noradrenaline, and thiocyanate there was discrepancy in predominant trends between clinical and experimental data. We found no clinical studies to corroborate the considerable number of experimental data showing a toxic effect for cholate, cysteine, and glutarate. For 4-OH-hexanal, intercellular adhesion molecule 1 and malondialdehyde, experimental and clinical data uniformly pointed to a toxic impact.

DISCUSSION

The main conclusions of this hypothesis-generating analysis are as follows: (1) screening of 11 untargeted metabolomic analyses and 1 targeted review allowed selection of 103 molecules that had as of yet not been screened for their clinical or experimental effect; (2) when these molecules were checked for their beneficial, toxic, or neutral impacts, literature on any effect was not found for 55 molecules, 3 molecules had no significant effect, and 13 showed beneficial effects; (3) for 32 compounds, at least 1 report on a toxic effect was found, but for several of these molecules there were more reports of a benefit or a neutral impact than of a toxic effect; (4) the untargeted top-down approach yielded a larger proportion of molecules with a benefit than the targeted bottom-up approach; and (5) these findings underscore the need for therapeutic strategies for uremia that also consider the metabolites with a benefit.

Among the substances with the highest support for toxicity, malondialdehyde and 4-OH-hexanal can be classified as reactive carbonyl species which are the result of oxidative stress, whereas carboxymethyllysine is a glycation product. All 3 substances are involved in post-translational modifications which induce functional pathophysiologic mechanisms, among which pro-inflammatory and vascular damaging effects play a prominent role.[27-30] This is an illustration of the biological importance of post-translational modifications in the uremic syndrome although this high yield may be biased in part by a higher scientific interest, emanating in a larger number of studies providing evidence than for other solutes[10] and/or by publication bias.

Proportionally more toxic solutes were selected by the targeted bottom-up approach than by the untargeted top-down metabolomics approach. However, the top-down approach is likely to provide a more balanced view of the reality of uremic retention, with a greater proportion of substances for which no toxicity was found.

It might be hypothesized that the concentration of the substances showing a benefit would be decreased in CKD, in this way adding to the harm caused by uremia. However, for 6 of the 13 beneficial compounds, the metabolomic studies did not report on the direction of the concentration changes. For the remaining 7 compounds, the serum concentration was increased. This finding confirms a previously raised hypothesis of dualism among uremic retention solutes[31] whereby elementary metabolic pathways not only produce toxins but also beneficial substances. If those are retained in CKD together with the toxins, this evolution may neutralize in part the pathophysiologic effects of uremic toxicity. In line with this suggestion of duality, a study in individuals aged older than 65 years with estimated glomerular filtration rate (eGFR) below 20 mL/min/1.73 m^2 showed that high 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid levels conferred an independent health benefit by possibly counteracting the unfavorable association between trimethylamine-N-oxide and outcomes.[32]

These observations could be relevant for the conceptualization of future extracorporeal removal strategies for uremic toxins, which currently do not discern for their removal pattern between toxic and beneficial compounds. This is an aspect that urgently needs to be investigated, together with the development of strategies avoiding or compensating for this indiscriminative solute removal, e.g., by preserving kidney function, restoring intestinal metabolic balance, developing bioartificial organs, and/or applying regenerative medicine.[33] In addition, these observations stress the necessity for metabolomic studies to report both the relationship of solutes with outcomes and the direction of the corresponding concentration changes versus normal or versus a higher GFR. For the current analysis, this was only the case for 6 out of 11 studies.[15,16,19,20,23,24]

Some molecules deserve careful consideration of the findings as described in detail in the supplementary file (Item S3: Supplemental Data – Extended Discussion). For neopterin, convincing experimental data indicated a benefit[34] in contrast to observational clinical data associating neopterin to negative outcomes.[35] For citric acid, experimental and clinical studies were discrepant.[36,37] In addition, when toxicity was demonstrated, the applied experimental doses were particularly high,[36] resulting in concentrations by far exceeding those in CKD.[38] For propionic acid, administered doses in studies showing toxicity were extremely high[39] whereas lower doses showed a benefit.[39,40] Also for uracil, studies showing carcinogenicity[41] applied excessive doses compared with what is sufficient to increase plasma concentration in humans.[42] For myoglobin, doubts could be raised about the experimental conditions mimicking rhabdomyolysis,[43] which very likely are irrelevant for CKD.[8,44]

Substances for which the need for more extended study is acknowledged are choline, anthranilic acid, and pentosidine. For choline and anthranilic acid, this is because of their established role in pathologic metabolic pathways of CKD,[31,45] as corroborated by data analysis outside the study timeframe.[46,47] For pentosidine, we collected sufficient clinical data supporting a pathophysiologic role,[48,49] but this could not be corroborated by experimental data. Those were, however, found outside the preset timeframe,[50] and, based on clinical studies showing that drugs increasing bone strength, are associated with the lowering of bone pentosidine.[51]

Some molecules appeared to be beneficial under certain conditions and for certain organs and toxic in other conditions. This is the case for noradrenaline,[52,53] IGF,[54,55] and VEGF.[56,57] These data suggest that there is not only a dualism with opposite effects among uremic retention solutes,[31] but also within individual compounds, as has also been observed earlier for some tryptophan metabolites like indole acetic acid and serotonin.[31]

The absence of data supporting toxicity for a given compound is not the same as evidence of nontoxicity. The solute may not have been studied at all or not been studied within the period of our literature search, and/or the restrictions imposed by our search method may have excluded relevant arguments. For example, for dimethylguanosine, our search found no data showing a pathophysiological impact. However, earlier studies showed that it inhibits Ca^{2+}-ATPase causing calcium accumulation in organs, particularly in the brain, with a potential detrimental effect on cognitive function.[58] Also, even if molecules are shown to be beneficial, their downstream metabolites may have a definite toxic effect. This is exemplified by N-methyl-2-pyridone-5-carboxamide (2PY), for which a toxic profile was demonstrated in previous studies.[59] Nevertheless, 2PY is a major metabolite of nicotinamide, which based on our collected data was classified as beneficial (supplemental data – beneficial effects and comments).

To the best of our knowledge, this is the first analysis, using a preset methodology, searching metabolomic studies for their yield in retrieving uremic toxins and comparing this yield to that of the classic targeted approaches. The analysis was also not only considering toxic effects, as usual, but also paid extensive attention to beneficial effects. Based on our findings, a few suggestions might be considered for the conduction of future metabolomic analyses in CKD as well as for their examination for clinical and experimental effects (Table 5). To facilitate

Table 5. Recommendations for Future Metabolomic Studies

- Preferably use both a detection and validation group.
- Study only outcomes/endpoints that are relevant to the population with CKD
  - Hard outcomes
    - Overall mortality
    - Cardiovascular events
    - Susceptibility to infection
    - Progression of kidney dysfunction, eGFR slope
    - Cognitive dysfunction
  - Patient-centered outcomes
    - Fatigue
    - Pruritus
    - Depression
    - Loss of appetite
    - Taste disturbances
    - Pain
    - Sensory disturbances
    - Muscle weakness
    - Cramps
    - Restless legs
- Reporting of studies based on untargeted metabolomics platforms
- Report changes in abundance vs. normal
- Standardization of approaches to allow comparison and biostatistical integration of different studies
  - Standardization of the pre-analytic approaches (collection; storage)
  - Standardization of the analytical approaches (targeted vs. untargeted; purification steps; use of internal standards; external calibration; quantification)
  - Optimization measures allowing data integration
- For those compounds with a significant correlation
  - Define whether correlation to outcomes is positive or negative
  - Check literature for potentially relevant toxic or beneficial effects (preferably related to the primary study outcomes but possibly also to other elements).
  - If nothing is found (or even if something is found), perform experimental studies of relevant outcomes for validation of causality (preferably related to the primary study outcomes but possibly also to other elements).
  - Report not only toxic effects but also beneficial or neutral effects
  - For scientific journals: handle studies reporting beneficial or neutral effects in a similar way as studies pointing to a toxic effect
- Define a protocol for studies examining toxic, beneficial or neutral clinical or experimental impact of molecules, retrieved by metabolomic analysis

Abbreviation: eGFR, estimated glomerular filtration rate.

comparison among studies, particularly the need for standardization of pre-analytical and analytical approaches with optimization measures allowing data integration should be emphasized. It would be useful that recommendations in this regard are elaborated by a group of experts.

This study has drawbacks. A selection was made among a large array of metabolomic studies in CKD. Although based on objective criteria, use of a different strategy might have resulted in a different list of substances. For a large number of solutes, no data on the biologic impact, either beneficial or toxic, were found, but this is only illustrative of our current lack of knowledge of the full scale of biologic changes in CKD.

Concentrations assessed in the experimental studies were not necessarily corresponding with those observed in CKD. However, many of the studies we selected were not undertaken with uremic toxicity or even CKD in mind, whereas for several molecules considered the uremic concentrations are not known. In addition, the biological effect of a given concentration of a molecule on its own (as is usually the case in toxicity studies) might be different from what it does in uremia, when it is retained together with other molecules. Ideally, uremic concentrations should be taken into account, but effects should, to our opinion, not necessarily be discarded if occurring at concentrations different from CKD or if those concentrations are not known. However, for some molecules, if the concentration used was an obvious source of bias, this is mentioned in the discussion and in Table 4.

Further bias may be created by the selection of molecules from metabolomic studies with and without validation processes, which might create a disbalance in molecule yield; the heterogeneity of the experimental studies considered; and the reliance on significance for inclusion of solutes and effects, which might differ depending on the number of analyzed data.

Clinical data were largely obtained from observational studies, which are hypothesis-generating and not exhibiting the highest level of evidence. It is of note, however, that it is difficult to organize controlled studies on removal of specific solutes in CKD, as most removal strategies (eg, dialysis) aim for several solutes at the same time. Consequently, observational data that are supported by experimental data pointing into the same direction are more relevant than if conclusions of experimental and clinical data are conflicting, like observed for neopterin.

In conclusion, this search for untargeted metabolomic studies next to the analysis of targeted approaches, allowed identifying several solutes that are potential uremic toxins. Of note, this study should essentially be seen as hypothesis-generating, in view of some inherent drawbacks of the selected studies, as summarized above. Several solutes with the highest toxicity evidence relate to post-translational modifications, underpinning the pathophysiologic potential of such changes. The metabolomic-based top-down approach provided a lower number of toxins than the traditional bottom-up approach, but offers a more balanced view of uremic retention. Our findings also underscore our lack of knowledge on the biological effects of most metabolomic changes observed in CKD.

Finally, by identifying compounds with a beneficial impact, metabolomics also corroborates previous suggestions of the dualism of the metabolic pathways leading to traditionally accepted uremic toxins, which could result in a change in therapeutic paradigm.

SUPPLEMENTARY MATERIALS

Supplementary File (PDF)

Figure S1: Overview of experimental (A) and clinical (B) results per substance and per organ/pathophysiological system of interest. Molecules for which no studies were available were not included. The number of concerned studies is mentioned in the blocks. Studies showing no toxic effect before the slash; studies showing a toxic impact after the slash. The blocks are colored blue when the majority of findings per organ/pathophysiological system was beneficial or neutral, red if the majority was toxic, and orange in case of a tie. Abbreviations: Syst, system.

Item S1: Supplemental data – summary search results.

Item S2: Beneficial effects and comments.

Item S3: Supplemental data – extended discussion.

Table S1: Summary Table of Studies.

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