



Review Article

Gut-derived uremic toxins and cardiovascular health in chronic kidney disease

Ming-Chun Chen^{a,b}, Chiu-Huang Kuo^{c,d}, Yu-Li Lin^{b,d}, Bang-Gee Hsu^{b,d*}

^aDepartment of Pediatrics, Hualien Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Hualien, Taiwan, ^bSchool of Medicine, Tzu Chi University, Hualien, Taiwan, ^cSchool of Post-Baccalaureate Chinese Medicine, Tzu Chi University, Hualien, Taiwan, ^dDivision of Nephrology, Hualien Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Hualien, Taiwan

Submission : 08-Nov-2024
Revision : 01-Jan-2025
Acceptance : 15-Jan-2025
Web Publication : 11-Apr-2025

ABSTRACT

Uremic toxins (UTs) are bioactive compounds that accumulate in chronic kidney disease (CKD) due to impaired renal clearance, exacerbating disease progression and cardiovascular (CV) complications. These toxins originate from endogenous metabolism, gut microbiota, and dietary intake and include protein-bound UTs such as p-cresyl sulfate, indoxyl sulfate, and indole acetic acid, as well as small, water-soluble toxins such as trimethylamine-N-oxide and phenylacetylglutamine. Their accumulation promotes oxidative stress, inflammation, and endothelial dysfunction, contributing to vascular damage and associated with CV risk. Current management strategies focus on dietary interventions, prebiotics, probiotics, oral sorbents, emerging pharmacological approaches, and advanced dialysis techniques, but clinical outcomes remain inconsistent. Recent trials have demonstrated the potential of agents such as sevelamer, high-amylose-resistant starch, and AST-120 to reduce UT levels and improve certain vascular markers. However, more robust, long-term studies are necessary to fully establish the therapeutic efficacy and optimize treatment strategies to mitigate the impact of gut-derived UTs on CKD and CV health.

KEYWORDS: *Chronic kidney disease, Gut-derived uremic toxins, Indole acetic acid, Indoxyl sulfate, p-cresyl sulfate, Phenylacetylglutamine, Trimethylamine-N-oxide*

INTRODUCTION

Uremic toxins (UTs) are a diverse group of compounds that accumulate in the body due to impaired renal function, contributing to the progression of chronic kidney disease (CKD) and end-stage renal disease (ESRD). These toxins originate from endogenous metabolic processes, microbial metabolism in the gut, and dietary intake. Accurate classification of UTs is essential for understanding their role in disease progression and for developing effective therapeutic strategies [1].

Previously, UTs were classified based on their physicochemical properties into four categories: free water-soluble low-molecular-weight solutes (<500 Da), protein-bound water-soluble low-molecular-weight solutes (<500 Da), middle-molecular-weight solutes (500–12,000 Da), and high-molecular-weight solutes (>12,000 Da) [2]. However, a more recent classification proposed by Rosner *et al.* offers a nuanced six-category model, which further stratifies UTs based on size, protein binding, and clearance challenges. The proposed categories range from small protein-bound molecules (<500 Da) to large molecules (>58,000 Da), each presenting unique challenges for dialysis and clinical management [3].

Gut-derived UTs are a significant subset of pathological solutes that accumulate in the body due to impaired renal clearance, contributing to the progression of CKD and associated complications such as cardiovascular disease (CV) and systemic inflammation [4]. These UTs are primarily produced through microbial metabolism in the gut, where bacteria ferment dietary proteins and amino acids, generating precursors that are absorbed and metabolized into UT [4]. Notable examples include p-cresyl sulfate (pCS), indoxyl sulfate (IS), and indole acetic acid (IAA), which are protein-bound UTs (PBUTs), are produced by gut microbiota metabolism of dietary proteins and are difficult to remove through conventional dialysis due to their protein-binding properties [5,6]. Other gut-derived UTs are small, water-soluble UTs, such as trimethylamine-N-oxide (TMAO) and phenylacetylglutamine (PAGln), that are readily absorbed into circulation but poorly excreted in CKD, leading to systemic accumulation [6,7]. The accumulation of these gut-derived UTs contributes to multiorgan dysfunction through

*Address for correspondence: Dr. Bang-Gee Hsu,

Division of Nephrology, Hualien Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, 707, Section 3, Chung-Yang Road, Hualien, Taiwan.
E-mail: gee.lily@msa.hinet.net

Access this article online

Quick Response Code:



Website: www.tcmjmed.com

DOI: 10.4103/tcmj.tcmj_293_24

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: WKHLRPMedknow_reprints@wolterskluwer.com

How to cite this article: Chen MC, Kuo CH, Lin YL, Hsu BG. Gut-derived uremic toxins and cardiovascular health in chronic kidney disease. *Tzu Chi Med J* 2025;37(3):264-74.

inflammatory, oxidative stress, and apoptosis pathways, promoting immune dysfunction, vascular calcification, kidney fibrosis, and CV events in CKD patients [6]. However, clinical studies investigating interventions such as oral activated charcoal (AST-120), prebiotics, and probiotics to reduce UTs and its toxic metabolites or restore gut microbial balance have not demonstrated significant effects on CV outcomes or CKD progression, underscoring the need for more robust research to develop effective therapies for reducing gut-derived UTs and improving CKD management [8-13].

This review aims to provide a comprehensive overview of the impact of gut-derived UTs associated with vascular complications in CKD. By examining the metabolic pathways, mechanisms of toxicity, and clinical evidence linking these UTs to adverse health outcomes, the review seeks to highlight current gaps in research and therapeutic management. In addition, it explores existing and emerging strategies to reduce UTs levels, emphasizing the need for innovative approaches to improve vascular health and overall patient outcomes in CKD.

METABOLIC PATHWAY OF GUT-DERIVED UREMIC TOXINS

Gut-derived UTs originate from the metabolism of dietary amino acids by gut bacteria. In the liver, these microbial metabolites undergo various modifications, such as sulfation or conjugation, enhancing their water solubility for renal excretion. Understanding these metabolic pathways underscores the interconnected roles of diet, microbial metabolism, and renal function in CKD progression and highlights the need for targeted therapeutic strategies. Table 1 and Figure 1 summarize the metabolic pathways of gut-derived UTs.

pCS is a PB UTs derived from the metabolism of tyrosine by gut microbiota. In the colon, anaerobic bacteria metabolize tyrosine from dietary proteins, producing p-cresol, a precursor of pCS. This p-cresol is absorbed through the intestinal wall, with factors such as gut dysbiosis and increased intestinal permeability in CKD patients enhancing its absorption. Once in circulation, p-cresol undergoes sulfation in the liver, catalyzed by sulfotransferase enzymes (SULTs), which add a sulfate group to form the more water-soluble pCS. In healthy individuals, pCS is excreted by the kidneys; however, in CKD, impaired renal function leads to reduced clearance and the accumulation of pCS in the bloodstream. Its strong binding to plasma proteins, particularly albumin, limits removal through

standard dialysis methods, contributing to its persistence and systemic toxicity [5,14].

IS is a PB UTs derived from the metabolism of tryptophan by gut microbiota, known to accumulate in patients with CKD due to impaired renal clearance. The metabolic pathway begins with tryptophan, an essential amino acid from dietary proteins metabolized by anaerobic bacteria, including *Bacteroides* and *Clostridia* species, into indole in the large intestine. Indole is absorbed into the portal circulation and transported to the liver, where it undergoes transformation by cytochrome P450 enzymes into indoxyl. Indoxyl is subsequently conjugated with a sulfate group by SULTs, producing IS. Under normal conditions, IS is eliminated by the kidneys through glomerular filtration and tubular secretion; however, in CKD patients, impaired renal function reduces IS clearance, leading to its systemic accumulation [5,15].

IAA is a gut-derived UTs that results from the metabolism of tryptophan and is linked to CV complications in CKD. The metabolic pathway begins in the gut, where tryptophan, an essential amino acid from dietary proteins, is metabolized by anaerobic bacteria, such as *Bacteroides* and *Clostridia* species, into indole. Indole is absorbed through the intestinal wall into the bloodstream and transported to the liver via the portal circulation. In the liver, indole undergoes oxidation by cytochrome P450 enzymes into indole-3-acetaldehyde, which is subsequently oxidized by aldehyde dehydrogenase (ALDH) to form IAA. In healthy individuals, IAA is excreted through the kidneys; however, in CKD patients, reduced renal function impairs IAA clearance, leading to its accumulation in the bloodstream [5,16].

The metabolic process of TMAO begins with ingesting dietary compounds such as choline, carnitine, and betaine, commonly found in foods such as red meat, eggs, fish, and dairy products. In the gut, these compounds are metabolized by bacteria – primarily from the *Enterobacteriaceae*, *Clostridia*, and *Lachnospiraceae* families – into trimethylamine (TMA), a precursor that plays a critical role in TMAO synthesis. TMA is absorbed into the bloodstream and transported via the portal circulation to the liver, where it undergoes oxidation by flavin-containing monooxygenase 3, resulting in the formation of TMAO. This oxidation enhances TMAO's water solubility, enabling its excretion through the kidneys under normal conditions. However, impaired renal function in CKD reduces TMAO clearance, leading to its accumulation in the bloodstream [5,17].

Table 1: The metabolic pathways of gut-derived uremic toxins

Toxin	Precursor	Gut microbial metabolism	Liver conversion	Excretion	Class
pCS	Tyrosine	Tyrosine→p-cresol	p-cresol→pCS (sulfation)	Renal excretion, impaired in CKD	Protein-bound
IS	Tryptophan	Tryptophan→indole	Indole→indoxyl→IS (sulfation)	Renal excretion, impaired in CKD	Protein-bound
IAA	Tryptophan	Tryptophan→indole	Indole→indole-3-acetaldehyde→IAA (oxidation)	Renal excretion, impaired in CKD	Protein-bound
TMAO	Choline, carnitine, betaine	Choline, carnitine, betaine→TMA	TMA→TMAO (oxidation by FMO ₃)	Renal excretion, impaired in CKD	Water-soluble
PAGln	Phenylalanine	Phenylalanine→PAA	PAA→PAGln (conjugation with glutamine)	Renal excretion, impaired in CKD	Water-soluble

TMA: Trimethylamine, PAA: Phenylacetic acid, pCS: p-cresyl sulfate, IS: Indoxyl sulfate, IAA: Indole acetic acid, TMAO: TMA-N-oxide, PAGln: Phenylacetylglutamine, CKD: Chronic kidney disease

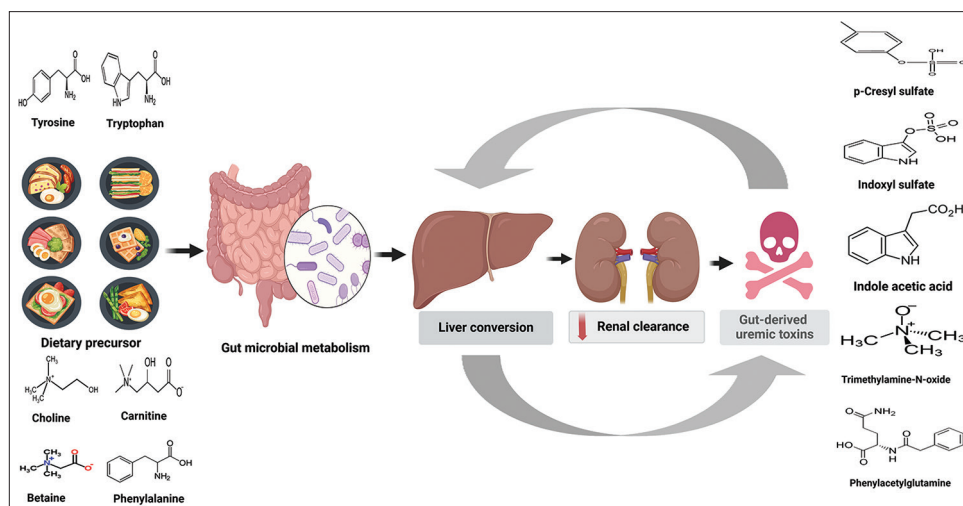


Figure 1: The metabolic pathways of gut-derived uremic toxins. Gut-derived uremic toxins are metabolic byproducts of dietary amino acids processed by intestinal microbiota. These microbial metabolites are then transported to the liver, where they undergo modifications, such as sulfation or conjugation, which increase their hydrophilicity and facilitate renal excretion. In cases of chronic kidney disease, however, impaired renal function reduces the clearance of these toxins, leading to their accumulation in the bloodstream and contributing to systemic toxicity. Created in BioRender.com

The metabolic pathway of PAGln originates from the dietary intake of phenylalanine, an essential amino acid found in protein-rich foods such as meat, fish, dairy, and legumes. In the gut, phenylalanine is metabolized by microbial enzymes, primarily from *Clostridia* species and other bacteria, into phenylacetic acid (PAA). Two distinct microbial pathways contribute to PAA synthesis. In the first pathway, L-amino acid deaminase converts phenylalanine into phenylpyruvic acid, which is further processed by phenylpyruvate-ferredoxin oxidoreductase into phenylacetyl-CoA, and subsequently into PAA by acetyl-CoA synthetase. In the alternative pathway, phenylpyruvate decarboxylase converts phenylpyruvic acid into phenylacetaldehyde oxidized by ALDH to form PAA. Following its absorption through the intestinal wall, PAA is transported to the liver via the portal circulation, where it undergoes conjugation with glutamine catalyzed by phenylacetyltransferase enzymes, producing PAGln. This conjugation enhances the solubility of PAA, facilitating its excretion by the kidneys. However, in individuals with CKD, reduced renal function impairs PAGln clearance, resulting in its accumulation in the bloodstream [7,18].

GUT-DERIVED UREMIC TOXINS AND CARDIOVASCULAR RISK IN CHRONIC KIDNEY DISEASE

CV diseases are the leading cause of death in CKD patients, and gut-derived UTs play a central role in accelerating atherosclerosis, endothelial dysfunction, and vascular calcification, all of which increase CV risk [19,20]. However, some studies show inconsistent results regarding gut-derived UTs and CV disease in CKD patients. Additional inquiries are required to elucidate these gut-derived UTs with CV disease findings in CKD patients.

pCS can directly induce cardiac dysfunction by promoting apoptosis of cardiomyocytes through the activation of

nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and increased reactive oxygen species (ROS) production, leading to endothelial dysfunction and vascular calcification [21,22]. Long-term exposure to pCS in CKD rats significantly increases aortic and peripheral artery calcification, activating inflammation and coagulation pathways [23]. A meta-analysis study noted that elevated free pCS levels are associated with increased all-cause mortality and risk of CV events in CKD patients, including dialysis patients [24]. In a cohort of 523 patients with nondialysis CKD stages 1–5 patients, free pCS showed a positive association with fatal and nonfatal CV events, even after adjusting for age, sex, blood pressure, diabetes, and estimated glomerular filtration rate (eGFR), while IS and IAA showed no significant association [25]. Higher serum pCS levels significantly increase the risk of CV events in patients with peritoneal dialysis [26]. However, HEMO study found no significant associations with cardiac death, sudden cardiac death, or overall CV events with pCS levels in hemodialysis patients [27]. These contrasting results emphasize the necessity for further research to elucidate the role of pCS in CV outcomes and to reconcile the discrepancies observed in clinical settings.

IS exerts its effects via mechanisms such as the aryl hydrocarbon receptor (AhR), leading to the inhibition of nitric oxide (NO) production and the disruption of endothelial repair. IS exacerbates oxidative stress, incites inflammatory responses, and contributes to endothelial dysfunction, all of which are associated with the pathogenesis of atherosclerosis, vascular calcification, and thrombosis [16,28]. Lin *et al.* noted that elevated free IS level was significantly associated with all-cause mortality; conversely, free IS was not significantly associated with the incidence of CV events in a meta-analysis study in CKD patients [24]. Higher serum total IS levels were associated with increased all-cause mortality in hemodialysis patients in the Japan Dialysis Outcomes and Practice

Patterns Study [29]. Li *et al.* also noted that higher IS levels are associated with ventricular septal thickness and mitral regurgitation in 100 hemodialysis patients [30]. In a cohort of 523 patients with nondialysis CKD stages 1–5 patients, IS did not exhibit a statistically significant correlation with either fatal or nonfatal CV events [25]. However, the HEMO study revealed no statistically significant correlations with cardiac mortality, unexpected cardiac mortality, or overall CV occurrences in individuals undergoing hemodialysis with IS [27]. Chen *et al.* also noted that IS levels did not show an association with CV events in patients undergoing peritoneal dialysis [26]. The insignificant relationship between IS levels and fatal or nonfatal CV events may be attributed to several factors. Unmeasured confounding variables, such as other UT, genetic predispositions, or comorbid conditions, could dilute the observed impact of IS. Limitations in measurement methods for IS or CV events may also contribute to the lack of a significant association due to insufficient sensitivity or specificity. In addition, small sample sizes or study design constraints might result in insufficient statistical power to detect true relationships.

IAA promotes oxidative stress and inflammation in endothelial cells by activating the AhR pathway, which leads to upregulation of cyclooxygenase-2 (COX-2) and increased production of ROS [31]. IAA also induces tissue factor (TF) expression in human endothelial cells through the AhR and nuclear factor-kappa B (NF- κ B) pathways [16]. Higher serum IAA levels are associated with increased mortality and CV events in CKD patients [31]. In CKD patients, elevated free IAA predicts mortality and CV events; however, this association does not extend to individuals who have received kidney transplantation [32]. However, IAA failed to demonstrate a statistically significant association with both fatal and nonfatal CV events in patients with nondialysis CKD stage 1–5 [25]. Based on the available data, these findings underscore the complexity of IAA's role in CKD and related outcomes. The discrepancies may arise from differences in study populations, varying kidney function levels, or confounding factors. Further research is needed to clarify the mechanisms and contexts, in which IAA influences CV events and mortality.

TMAO contributes to CV disease by promoting cholesterol metabolism disruption, inflammation, endothelial dysfunction, and platelet activation [33,34]. A systematic review and a meta-analysis of 11 cohort studies found that higher TMAO levels are significantly associated with a 23% increased risk of CV events and a 55% increased risk of all-cause mortality [35]. In 737 advanced CKD with aged ≥ 65 years, those whose eGFR has decreased for the initial instance to ≤ 20 mL/min/1.73 m² during 6 months, higher TMAO levels are associated with an increased risk of all-cause mortality [36]. In 1243 maintenance hemodialysis patients with moderate-to-severe secondary hyperparathyroidism, there was no significant association between TMAO levels and CV or all-cause mortality [37]. Among 513 peritoneal dialysis patients, higher TMAO quartiles had a greater risk of developing peritonitis and higher CV disease mortality. However, there was no significant association between TMAO levels and all-cause mortality [38].

Elevated PAGln levels have been associated with obesity, increased atherosclerotic plaque burden, and type II diabetes [7]. PAGln influences CV disease by activating adrenergic receptors, which promote platelet thrombosis, inflammation, and oxidative stress [39]. In a 4000 participant cohort, high levels of PAGln are associated with an increased incidence of major adverse CV events over a 3-year period, even after accounting for traditional cardiac risk factors [40]. In incident 394 hemodialysis patients, higher levels of PAGln are linked to increased risks of CV mortality and first CV events [41]. High PAGln levels are positively associated with an increased risk of overall mortality and CV events, independent of kidney function and protein binding in 488 patients with CKD stages 1–5 [42]. However, the HEMO study found no significant associations between cardiac death, sudden cardiac death, or overall CV events with PAGln in hemodialysis patients [27].

Fibroblast growth factor-23 (FGF-23) and phosphate are emerging as key players in CV disease and mortality in CKD patients [43,44]. As kidney function declines, a reduction in nephron number and α -klotho expression in renal distal tubules impairs the action of FGF-23, leading to phosphate accumulation. To mitigate phosphate overload, remaining nephrons increase urinary phosphate excretion, driven by elevated secretion of FGF-23 and parathyroid hormone (PTH) from bone and parathyroid glands. This compensatory mechanism, historically termed uremic secondary hyperparathyroidism, includes FGF-23-mediated deactivation of calcitriol through upregulation of 25-hydroxyvitamin D-24-hydroxylase, which reduces calcium and phosphate absorption from the gut. Although PTH partially compensates by stimulating calcitriol production, this feedback system becomes ineffective as CKD progresses [45]. In CKD, declining kidney function reduces phosphate excretion, leading to elevated FGF-23 and hyperphosphatemia. Hyperphosphatemia and elevated FGF-23 levels emerge as critical factors contributing to CV complications, including hypertension, vascular calcification, and left ventricular hypertrophy [43]. FGF-23 elevation compensates for phosphate retention by downregulating type IIa/c sodium-dependent phosphate transporter (NaPi-2a/c) in the kidney but also directly promotes left ventricular hypertrophy via FGF receptor 4 (FGFR4)–calcineurin–nuclear factor of activated T-cells signaling. In addition, FGF-23 activates the renin-angiotensin-aldosterone system (RAAS) and increases sodium chloride co-transporter expression, contributing to hypertension. Hyperphosphatemia exacerbates vascular damage by inducing osteochondrogenic differentiation and releasing calcium/phosphate-loaded vesicles in vascular smooth muscle cells (VSMCs) through Pit-1, leading to vascular calcification. This phosphate-induced vascular calcification, combined with RAAS activation and sympathetic nervous system activity, further aggravates hypertension and CV complications in CKD [43]. Gut-derived UTs accumulate as a result of impaired renal clearance and increased gut absorption. Acting in synergy with elevated phosphate levels, gut-derived UTs alter the phenotype and function of VSMCs and endothelial cells, thereby promoting vascular calcification. This calcification is widespread and

predominantly affects the medial layer of blood vessels, extending beyond atherosclerotic plaques, contributing to the systemic vascular damage observed in CKD [46]. Gut-derived UTs play a critical role in systemic damage in CKD by stimulating RAAS activity, increasing ROS production, and promoting the release of inflammatory cytokines. These processes lead to oxidative and nonoxidative modifications of proteins, lipids, and DNA, which contribute to endothelial dysfunction, vascular calcification, and the development of hypertension, insulin resistance, and atherosclerosis [47]. Acting synergistically with hyperphosphatemia and FGF-23, gut-derived UTs exacerbate oxidative stress and systemic inflammation, further promoting left ventricular hypertrophy and vascular damage. These interconnected mechanisms significantly elevate CV risk in CKD.

GUT-DERIVED UREMIC TOXINS AND ARTERIAL STIFFNESS IN CHRONIC KIDNEY DISEASE

Arterial stiffness is an early indicator of CV disease risk, particularly in CKD patients [48]. CKD patients experience increased arterial stiffness due to traditional CV risk factors and CKD-specific factors, such as abnormalities in calcium, phosphorus, PTH, and UTs, leading to vascular calcification [49,50]. This phenomenon of arterial stiffness transpires when blood vessels experience a reduction in elasticity attributable to structural alterations, including the accumulation of collagen and the degradation of elastin, which are further aggravated by elements such as oxidative stress, inflammation, and deranged mineral metabolism. The resultant structural modifications culminate in elevated central blood pressure, increased pulse pressure, and additional strain on the heart and other organ systems, thereby significantly raising the risk of heart failure, stroke, and renal damage [50]. Several measurement techniques are available for arterial stiffness. Carotid-femoral pulse wave velocity (cfPWV) is the gold standard for measuring aortic stiffness and predicts CV risk, but it requires technical expertise and is influenced by blood pressure [51]. Brachial-ankle pulse wave velocity (baPWV) is simple, reproducible, and operator independent to assess both central and peripheral arterial stiffness; however, it is influenced by blood pressure and may not provide an accurate arterial stiffness in a leg exhibiting a diminished ankle-brachial index (ABI) [52,53]. Furthermore, it is also subject to variations due to body size and the positioning of the body during the measurement process [53]. The cardio-ankle vascular index (CAVI), which operates independently of blood pressure, delivers a reliable quantification of arterial stiffness extending from the aorta and femoral artery to the tibial artery; however, it necessitates specialized apparatus that may not be broadly accessible given that a substantial portion of the investigations surrounding CAVI has been undertaken within Asian cohorts, and its computation is more intricate than that of simpler metrics. Furthermore, recent studies have indicated that CAVI might not be as pressure-independent as initially thought, leading to the development of CAVI0, which potentially provides a more

refined assessment of pressure-independent assessment of arterial stiffness [54,55].

Several clinical studies have demonstrated that gut-derived UTs contribute to arterial stiffness in CKD and hemodialysis patients. In 149 stage 3–4 CKD patients, pCS levels were significantly associated with increased arterial stiffness measured by cfPWV, while IS showed no significant association [56]. In a cohort of 155 CKD stages 1–5 patients, IS, age, and systolic blood pressure were independent predictors of arterial stiffness measured by cfPWV [57]. Among 160 nondialysis stage 3–5 CKD patients, higher pCS levels were linked to increased arterial stiffness measured by baPWV, and age and diastolic blood pressure were also significant predictors [58]. In 118 HD patients, elevated pCS levels were correlated with increased arterial stiffness measured by cfPWV, along with diabetes mellitus and higher systolic blood pressure [59]. In addition, higher TMAO levels were associated with arterial stiffness measured by baPWV or cfPWV in both 157 nondialysis CKD patients and those 115 patients undergoing HD, with age and systolic blood pressure consistently emerging as significant predictors [60,61]. Among the 160 participants undergoing chronic peritoneal dialysis, serum TMAO level, age, and waist circumference as independent predictors of arterial stiffness measured by cfPWV [62]. In 100 kidney transplant recipients, along with age, systolic blood pressure, and fasting glucose levels, PAGln were also identified as independent factors associated with arterial stiffness measured by cfPWV [63]. These findings highlight the complex interplay between gut-derived UTs and traditional risk factors in the progression of arterial stiffness across various CKD stages, hemodialysis, peritoneal dialysis, and kidney transplantation populations. However, more research is needed to confirm the association between gut-derived UTs and arterial stiffness in CKD patients.

GUT-DERIVED UREMIC TOXINS AND ENDOTHELIAL FUNCTION IN CHRONIC KIDNEY DISEASE

Gut-derived UTs accumulate in CKD patients and contribute to endothelial dysfunction through multiple mechanisms, including the AhR, NF- κ B, mitogen-activated protein kinase (MAPK), and advanced glycation end products (AGEs)/receptor for advanced glycation end products (RAGE) pathways [64,65]. Upon activation, AhR translocates to the nucleus, forms a dimer with aryl hydrocarbon receptor nuclear translocator, and induces the expression of target genes such as CYP1A1, CYP1B1, and AhRR via its genomic pathway. In addition, AhR stimulates nongenomic pathways, including the activation of AP-1, which upregulates E-selectin expression. Gut-derived UTs also activate MAPK pathways, including p38MAPK and ERK1/2, through phosphorylation. Both AhR and MAPK pathways can further activate the NF- κ B signaling cascade. In uremic conditions, degradation of I κ B facilitates the translocation of NF- κ B subunits p50 and p65 to the nucleus, promoting the expression of pro-inflammatory and pro-thrombotic mediators such as monocyte chemoattractant protein-1, intercellular adhesion molecule-1, cyclooxygenase-2, and TF, which facilitate leukocyte recruitment and adhesion,

perpetuating vascular inflammation. These interconnected pathways amplify inflammatory responses and vascular injury, ultimately contributing to endothelial dysfunction in CKD [64]. Gut-derived UTs stimulate oxidative stress by disrupting mitochondrial function and activating NADPH oxidase, leading to the overproduction of ROS. AGEs mediate oxidative stress and impair vasoreactivity by reducing endothelial NO synthase activation in endothelial cells. ROS further amplifies endothelial inflammation by upregulating the surface expression of chemokines and adhesion molecules. These changes promote leukocyte recruitment and adhesion, exacerbating vascular inflammation. ROS reacts with NO, forming peroxynitrite, a highly reactive compound that depletes NO bioavailability, impairing endothelial-dependent vasodilation [64,65]. Moreover, gut-derived UTs induce the release of EMPs and alter microRNA regulation, contributing to inflammation, thrombosis, and vascular damage [64]. Gut-derived UTs induce cytoskeletal remodeling and reduce the expression of vascular endothelial (VE)-cadherin at the gene level. Furthermore, they activate Src kinase, which phosphorylates VE-cadherin, promoting its internalization and weakening cell-cell adhesion. Simultaneously, gut-derived UTs decrease the levels of ZO-1, a critical tight junction protein, further compromising the integrity of endothelial barriers. Together, these processes lead to the loss of intercellular junctions, enhancing endothelial permeability and facilitating the infiltration of inflammatory mediators and macromolecules into the subendothelial space, thereby contributing to vascular inflammation and endothelial dysfunction in CKD [64].

Most clinical studies elucidate the significant impact of IS or pCS on endothelial function across various stages of CKD. Rossi *et al.* conducted a comprehensive analysis involving 327 participants with kidney function stratified into four groups: normal kidney function ($n = 42$), nondialysis CKD ($n = 198$), hemodialysis ($n = 54$), and peritoneal dialysis ($n = 33$). The study demonstrated that both total and free serum concentrations of IS and pCS were significantly associated with endothelial function, as measured by flow-mediated dilation. These associations remained robust even after adjusting for traditional CV risk factors [66]. Kamiński *et al.* investigated the relationship between IS and markers of endothelial dysfunction in 51 patients with non-dialysis CKD stages 1–5. Their findings revealed that IS was independently associated with markers indicative of impaired endothelial function, including thrombomodulin and adhesion molecules [67]. Wang *et al.* examined the link between IS levels, diastolic blood pressure, and endothelial function in a cohort of 110 patients with nondialysis CKD stages 3–5. The results indicated that elevated IS concentrations, alongside increased diastolic blood pressure, were significantly correlated with reduced endothelial function, assessed via the vascular reactivity index [68]. Hobson *et al.* evaluated the effects of kidney transplantation on endothelial function in 27 CKD patients undergoing living-donor kidney transplantation. Despite observed improvements in vascular stiffness posttransplantation, endothelial function deteriorated. Furthermore, baseline IS levels were positively associated with endothelial dysfunction, as measured by the reactive hyperemia index [69]. Current clinical research on the relationship between IAA, TMAO, and PAGln with endothelial function in CKD is notably

limited, with no extensive clinical studies directly addressing these associations. In addition, only one clinical study is available that investigates the impact of pCS on endothelial function. Given the growing evidence linking gut-derived UTs to vascular health, future research should prioritize large-scale, well-designed clinical studies to explore these relationships comprehensively. Such investigations could help elucidate the mechanisms by which these metabolites contribute to endothelial dysfunction and inform potential therapeutic strategies for improving vascular outcomes in patients with CKD.

GUT-DERIVED UREMIC TOXINS AND PERIPHERAL ARTERY OCCLUSIVE DISEASE IN CHRONIC KIDNEY DISEASE

Peripheral artery occlusive disease (PAOD) is a common and severe vascular complication in CKD, characterized by progressive arterial narrowing that reduces blood flow, particularly to the lower extremities [70,71]. The relationship between CKD and PAOD is rooted in overlapping and unique risk factors that accelerate vascular damage. Both conditions are influenced by traditional CV risk factors, such as hypertension, diabetes, and dyslipidemia, but CKD introduces additional elements such as mineral and bone disorders, metabolic imbalances, UTs, and chronic inflammation, all of which intensify the risk of PAOD [71]. Patients may present with symptoms ranging from intermittent claudication to critical limb ischemia, increasing their risk of amputations and CV events [51,70]. In a large cohort of over 4,354 patients treated with endovascular or open surgery for symptomatic PAOD at 35 German medical centers, findings show that CKD, particularly ESRD, is linked to significantly higher risks of mortality, major amputations, and adverse CV events within 12 months after surgery [72]. Diagnosis typically involves noninvasive tests like ABI and imaging techniques, while management focuses on lifestyle modifications, pharmacological interventions, and revascularization procedures to improve blood flow and prevent disease progression [70,71].

Higher levels of pCS and IS are significantly associated with lower ABI in 100 hemodialysis patients. Total pCS was also identified as a significant predictor of vascular access failure and correlated with higher frequencies of procedures like percutaneous transluminal angioplasty and thrombectomy in hemodialysis patients [73]. IS detrimentally influences endothelial progenitor cells, compromising their role in neovascularization and tissue regeneration. It also fosters thrombosis in dialysis vascular access via oxidative stress and TF elevation, thereby exacerbating PAOD outcomes [74]. Lin *et al.* followed 200 hemodialysis patients without PAOD over a median period of 6.5 years. It found that higher IS levels were significantly associated with an increased risk of developing symptomatic PAOD, even after adjusting for traditional risk factors such as age, smoking, diabetes, and cardiovascular disease [75]. Chiu *et al.* noted that of 75 hemodialysis patients, higher levels of IS and C-reactive protein (CRP) were significantly associated with PAOD, as indicated by a lower ABI [76]. Current research primarily focuses on the role of IS in PAOD among hemodialysis

patients, with limited clinical studies addressing pCS and no existing research on the clinical impact of IAA, TMAO, or PAGln. In addition, there is a lack of studies examining the effects of IS, pCS, IAA, TMAO, and PAGln on CKD, patients undergoing peritoneal dialysis, kidney transplantation, or dealing with PAOD. Future research should aim to fill these gaps by investigating the PAOD of these gut-derived UTs across various CKD populations. Figure 2 shows the gut-derived UTs linked to cardiovascular health in chronic kidney disease.

MANAGEMENT OF GUT-DERIVED UREMIC TOXINS IN CHRONIC KIDNEY DISEASE

Managing gut-derived UTs in CKD involves strategies to reduce their systemic accumulation and mitigate adverse effects. Approaches include dietary modifications, such as increasing dietary fiber to promote beneficial gut microbiota and limiting precursor-rich foods; the use of prebiotics, probiotics, or fecal microbiota transplantation to modulate gut bacteria; oral sorbents like oral-activated charcoal AST-120 to bind toxin precursors in the gut; and emerging pharmacological interventions targeting specific toxin-producing pathways; combining high-volume ultrafiltration with hemodialysis, utilizing sorbents to bind and remove toxins, and introducing arterial bloodline molecules that compete with UTs for protein binding; although their impact on CV outcomes remains inconclusive [8-13].

Randomized controlled trials exploring strategies to manage gut-derived UTs in CKD have provided valuable but varied insights. A meta-analysis examines the impact of biotic supplements, including prebiotics, probiotics, and synbiotics,

on CKD patients. Based on 23 randomized controlled trials involving 842 participants, the study highlights that biotic interventions significantly reduce markers of oxidative stress, such as malondialdehyde (MDA) and total antioxidative capacity (TAC), and lower inflammation markers like interleukin-6 (IL-6). Biotics also decrease pCS and IS, particularly in dialysis-dependent patients. However, biotics did not significantly affect renal function markers, such as eGFR or serum albumin, nor influence lipid profiles or IAA levels. Prebiotics appeared more effective than probiotics in improving certain biomarkers, such as blood urea nitrogen (BUN) and IL-6 [77]. A network meta-analysis examines the efficacy of probiotics, prebiotics, and synbiotics in improving inflammatory markers, UTs levels, and gastrointestinal symptoms in ESRD patients undergoing dialysis. Prebiotics demonstrated the highest effectiveness in reducing IL-6, necrosis factor- α (TNF- α), IS, MDA, and BUN, while synbiotics were superior in lowering CRP and endotoxin levels. Probiotics were the most effective in alleviating gastrointestinal symptoms [78]. Another systematic review and meta-analysis evaluated the impact of microbiota-driven therapies, including probiotics, prebiotics, and synbiotics, on circulating IS and pCS levels in CKD patients. Based on 14 randomized controlled trials involving 513 participants, the findings reveal that microbiota-driven therapy significantly reduces circulating pCS levels but does not have a statistically significant effect on IS levels. Subgroup analyses indicate that prebiotics and synbiotics are effective in reducing pCS levels, while probiotics show no significant impact on either toxin [79]. In hemodialysis patients, a meta-analysis evaluates the impact of probiotics, prebiotics, and synbiotics on UTs, inflammation, and oxidative stress in hemodialysis

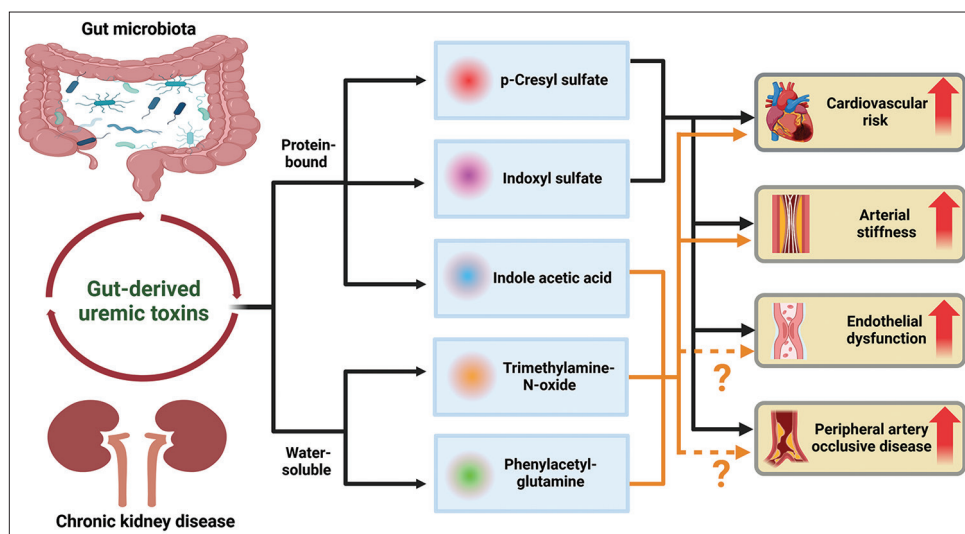


Figure 2: Gut-derived uremic toxins linked to cardiovascular health in chronic kidney disease. Protein-bound uremic toxins (PBUTs) are a group of toxins that are primarily produced in the gut and can build up in the body when kidney function is impaired, as in chronic kidney disease (CKD). Examples of these toxins include p-cresyl sulfate (pCS), indoxyl sulfate (IS), and indole acetic acid (IAA). These PBUTs bind to blood proteins, making them harder to remove during dialysis, leading to their accumulation in CKD patients. In addition to PBUTs, there are other types of uremic toxins (UTs), such as trimethylamine-N-oxide (TMAO) and phenylacetylglutamine (PAGln). Unlike PBUTs, these are small, water-soluble molecules easily absorbed into the bloodstream from the gut. However, in CKD, these water-soluble UTs are not efficiently filtered and eliminated by the kidneys, causing them to accumulate in the body. The accumulation of these UTs plays a significant role in the progression of cardiovascular disease and the development of arterial stiffness in patients with CKD. Among these UTs, research has mainly focused on pCS and IS, and these two UTs are known to contribute to endothelial dysfunction and peripheral artery occlusive disease (PAOD). However, less is understood about the clinical effects of other UTs like IAA, TMAO, and PAGln on endothelial dysfunction and PAOD in CKD, which are an area of interest for future research. Created in BioRender.com

patients. Based on data from 23 randomized controlled trials involving 931 participants, the results demonstrate that these biotic interventions significantly reduce circulating levels of pCS, endotoxins, and inflammatory markers such as CRP and IL-6. In addition, probiotics, prebiotics, and synbiotics enhance oxidative stress by increasing TAC and glutathione levels while reducing MDA [80]. A meta-analysis evaluates the effects of resistant starch supplementation on renal function and inflammatory markers in patients with CKD. The study analyzed 10 randomized controlled trials involving 355 participants and found that resistant starch supplementation significantly reduced levels of IS and BUN. However, no significant effects were observed on other inflammatory markers, such as IL-6 and TNF- α , or on levels of pCS, phosphorus, or serum albumin [81]. In a study assessing sevelamer ($n = 20$), a noncalcium phosphate binder, researchers observed a significant reduction in serum pCS levels compared to the calcium carbonate ($n = 20$) group; however, the intervention did not affect IS levels or improve vascular function (CAVI and ABI) and inflammation markers, such as high-sensitivity CRP [82]. Another 46 participants using fructooligosaccharide supplementation (12 g/day) or a placebo over 3 months showed a notable decrease in IL-6 and a nonsignificant trend toward lowering pCS. Although overall endothelial function, measured by flow-mediated dilation, did not show significant improvement [83]. High-amylose-resistant starch (RS2) supplementation over 16 weeks in 68 patients with stage G3a-G4 CKD led to favorable shifts in gut microbiota, including an increase in butyrate-producing bacteria and a reduction in *Bacteroides*, which correlated with a decrease in pCS. Despite these microbiota changes, RS2 did not significantly influence inflammation, oxidative stress, or arterial stiffness (cfPWV) [84]. A 24-week treatment with AST-120 improved endothelial function as evidenced by increased flow-mediated dilation and ROS markers in 40 CKD patients [85]. Over 6 months, 28 hemodialysis patients were divided into two groups: one on control and the other on AST-120 (6 g/day). AST-120 significantly improved microvascular endothelial function, as measured by laser Doppler flowmetry using iontophoresis of acetylcholine at baseline and again at 3 and 6 months in patients undergoing hemodialysis. However, improvements in macrovascular endothelial function measured by flow-mediated dilation were insignificant [86]. A network meta-analysis evaluates the efficacy of AST-120 in managing CKD. By analyzing 15 randomized controlled trials involving 3,763 patients, the study reveals that tailored or middle-dose AST-120 (approximately 6 g/day) significantly reduces the risk of composite renal outcomes and progression to ESRD compared to no treatment. While AST-120 did not show consistent improvements in acute renal function markers, it demonstrated a favorable impact on serum creatinine slope and IS levels. However, high-dose AST-120 (9 g/day) was less effective, potentially due to dose-related adverse effects impacting patient adherence [87]. Over 6 months, a low-protein diet (0.6–0.8 g/day) supplemented with ketoanalogues (KAs, 6 tab/day) led to significant reductions in both IS and pCS levels and improved endothelial function, as measured by flow-mediated dilation [88]. A review paper about advanced dialysis

techniques *in vitro*, preclinical, and clinical settings have demonstrated significant improvements in PB UTs removal using binding competitors such as tryptophan, fatty acids, and ibuprofen. In addition, *in silico* analyses suggest that binding competition outperforms other advanced dialysis modalities, including hemodiafiltration and adsorption techniques. Despite its promise, challenges remain, including selecting optimal binding competitors with minimal side effects and ensuring long-term safety [89]. These studies highlight the potential of various interventions to reduce UTs and improve vascular health in CKD, emphasizing the need for long-term research to establish the clinical benefits and optimize management strategies for toxin reduction and CV protection.

CONCLUSION

Gut-derived UTs play a critical role in the progression of CKD and its associated vascular complications. Despite advancements in understanding their metabolic pathways and the mechanisms by which they contribute to oxidative stress, inflammation, and endothelial dysfunction, current management strategies have shown mixed clinical outcomes. Dietary modifications, prebiotics, probiotics, oral sorbents such as AST-120, and advanced dialysis techniques offer potential benefits but require further investigation to establish long-term efficacy. Current research on gut-derived UTs and their CV risks in CKD is limited by short study durations, small sample sizes, and a lack of data linking toxin reduction to meaningful clinical outcomes, such as decreased CV events or mortality. Inconsistent findings and challenges in quantifying the levels of gut-derived UTs further complicate the understanding of their impact. In addition, the focus on single interventions without considering multimodal approaches neglects the complexity of the pathophysiology of gut-derived UTs. Future research should prioritize long-term, large-scale trials to assess the efficacy of combined interventions, including dietary modifications, pharmacological agents, and enhanced dialysis techniques. Mechanistic studies are needed to elucidate pathways linking gut-derived UTs to vascular calcification, endothelial dysfunction, and atherosclerosis while exploring personalized strategies based on genetic and microbiota variability. Improved measurement techniques, cost-effectiveness analyses, and investigations into early-stage CKD and non-CV outcomes will enhance the development of comprehensive strategies to mitigate gut-derived UTs-related risks, improve vascular health, and enhance the quality of life for CKD patients.

Data availability statement

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Financial support and sponsorship

This study was supported by a grant from Hualien Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation (TCMF-A 111-02), and the Ministry of Science and Technology (MOST 111-2314-B-303-015-MY3) in Taiwan.

Conflict of interest

Dr. Bang-Gee Hsu, an editorial board member at *Tzu Chi Medical Journal*, had no role in the peer review process or decision to publish this article. The other authors declared no conflicts of interest in writing this paper.

REFERENCES

1. Yavuz A, Tetta C, Ersoy FF, D'intini V, Ratanarat R, De Cal M, et al. Uremic toxins: A new focus on an old subject. *Semin Dial* 2005;18:203-11.
2. Kashani K, Cozzolino MG, Massy ZA, Blankestijn PJ, Stenvinkel P, Rosner MH, et al. Proposal for a new classification of solutes of interest in uremia and hemodialysis. *Blood Purif* 2023;52:233-41.
3. Rosner MH, Reis T, Husain-Syed F, Vanholder R, Hutchison C, Stenvinkel P, et al. Classification of uremic toxins and their role in kidney failure. *Clin J Am Soc Nephrol* 2021;16:1918-28.
4. Vanholder R, Glorieux G. The intestine and the kidneys: A bad marriage can be hazardous. *Clin Kidney J* 2015;8:168-79.
5. Graboski AL, Redinbo MR. Gut-derived protein-bound uremic toxins. *Toxins (Basel)* 2020;12:590.
6. Glorieux G, Gryp T, Perna A. Gut-derived metabolites and their role in immune dysfunction in chronic kidney disease. *Toxins (Basel)* 2020;12:245.
7. Cuervo L, McAlpine PL, Olano C, Fernández J, Lombó F. Low-molecular-weight compounds produced by the intestinal microbiota and cardiovascular disease. *Int J Mol Sci* 2024;25:10397.
8. Ramezani A, Raj DS. The gut microbiome, kidney disease, and targeted interventions. *J Am Soc Nephrol* 2014;25:657-70.
9. Lau WL, Savo J, Nakata MB, Vaziri ND. Altered microbiome in chronic kidney disease: Systemic effects of gut-derived uremic toxins. *Clin Sci (Lond)* 2018;132:509-22.
10. Laville SM, Massy ZA, Kamel S, Chillon JM, Choukroun G, Liabeuf S. Intestinal chelators, sorbants, and gut-derived uremic toxins. *Toxins (Basel)* 2021;13:91.
11. Melekoglu E, Samur FG. Dietary strategies for gut-derived protein-bound uremic toxins and cardio-metabolic risk factors in chronic kidney disease: A focus on dietary fibers. *Crit Rev Food Sci Nutr* 2023;63:3994-4008.
12. Sumida K, Pierre JF, Yuzefpolskaya M, Colombo PC, Demmer RT, Kovesdy CP. Gut microbiota-targeted interventions in the management of chronic kidney disease. *Semin Nephrol* 2023;43:151408.
13. Beker BM, Colombo I, Gonzalez-Torres H, Musso CG. Decreasing microbiota-derived uremic toxins to improve CKD outcomes. *Clin Kidney J* 2022;15:2214-9.
14. Gryp T, Vanholder R, Vaneechotte M, Glorieux G. p-cresyl sulfate. *Toxins (Basel)* 2017;9:52.
15. Leong SC, Sirich TL. Indoxyl sulfate-review of toxicity and therapeutic strategies. *Toxins (Basel)* 2016;8:358.
16. Addi T, Dou L, Burtey S. Tryptophan-derived uremic toxins and thrombosis in chronic kidney disease. *Toxins (Basel)* 2018;10:412.
17. Moraes C, Fouque D, Amaral AC, Mafra D. Trimethylamine N-oxide from gut microbiota in chronic kidney disease patients: Focus on diet. *J Ren Nutr* 2015;25:459-65.
18. Zhu Y, Dwidar M, Nemet I, Buffa JA, Sangwan N, Li XS, et al. Two distinct gut microbial pathways contribute to meta-organismal production of phenylacetylglutamine with links to cardiovascular disease. *Cell Host Microbe* 2023;31:18-32.e9.
19. Lim YJ, Sidor NA, Tonial NC, Che A, Urquhart BL. Uremic toxins in the progression of chronic kidney disease and cardiovascular disease: Mechanisms and Therapeutic Targets. *Toxins (Basel)* 2021;13:142.
20. El Chamieh C, Liabeuf S, Massy Z. Uremic toxins and cardiovascular risk in chronic kidney disease: What have we learned recently beyond the past findings? *Toxins (Basel)* 2022;14:280.
21. Han H, Zhu J, Zhu Z, Ni J, Du R, Dai Y, et al. p-cresyl sulfate aggravates cardiac dysfunction associated with chronic kidney disease by enhancing apoptosis of cardiomyocytes. *J Am Heart Assoc* 2015;4:e001852.
22. Watanabe H, Miyamoto Y, Enoki Y, Ishima Y, Kadowaki D, Kotani S, et al. p-cresyl sulfate, a uremic toxin, causes vascular endothelial and smooth muscle cell damages by inducing oxidative stress. *Pharmacol Res Perspect* 2015;3:e00092.
23. Opdebeeck B, Maudsley S, Azmi A, De Maré A, De Leger W, Meijers B, et al. Indoxyl sulfate and p-cresyl sulfate promote vascular calcification and associate with glucose intolerance. *J Am Soc Nephrol* 2019;30:751-66.
24. Lin CJ, Wu V, Wu PC, Wu CJ. Meta-analysis of the associations of p-cresyl sulfate (PCS) and indoxyl sulfate (IS) with cardiovascular events and all-cause mortality in patients with chronic renal failure. *PLoS One* 2015;10:e0132589.
25. Glorieux G, Vanholder R, Van Biesen W, Pletinck A, Schepers E, Neiryck N, et al. Free p-cresyl sulfate shows the highest association with cardiovascular outcome in chronic kidney disease. *Nephrol Dial Transplant* 2021;36:998-1005.
26. Chen Z, Xu J, Xing X, Xue C, Luo X, Gao S, et al. p-cresyl sulfate predicts clinical outcomes in sustained peritoneal dialysis: A 5-year follow-up cohort study and meta-analysis. *Ren Fail* 2022;44:1791-800.
27. Shafi T, Sirich TL, Meyer TW, Hostetter TH, Plummer NS, Hwang S, et al. Results of the HEMO study suggest that p-cresol sulfate and indoxyl sulfate are not associated with cardiovascular outcomes. *Kidney Int* 2017;92:1484-92.
28. Lano G, Burtey S, Sallée M. Indoxyl sulfate, a uremic endotheliotoxin. *Toxins (Basel)* 2020;12:229.
29. Yamamoto S, Fuller DS, Komaba H, Nomura T, Massy ZA, Bieber B, et al. Serum total indoxyl sulfate and clinical outcomes in hemodialysis patients: Results from the Japan dialysis outcomes and practice patterns study. *Clin Kidney J* 2021;14:1236-43.
30. Li Z, Ke G, Song L, Huang J, Zhang Y, Xiao J, et al. Association between cardiac outcomes and indoxyl sulfate levels in hemodialysis patients: A cross-sectional study. *Kidney Blood Press Res* 2022;47:239-46.
31. Dou L, Sallée M, Cerini C, Poitevin S, Gondouin B, Jourde-Chiche N, et al. The cardiovascular effect of the uremic solute indole-3 acetic acid. *J Am Soc Nephrol* 2015;26:876-87.
32. Liabeuf S, Laville SM, Glorieux G, Cheddani L, Brazier F, Titeca Beauport D, et al. Difference in profiles of the gut-derived tryptophan metabolite indole acetic acid between transplanted and non-transplanted patients with chronic kidney disease. *Int J Mol Sci* 2020;21:2031.
33. Tomlinson JA, Wheeler DC. The role of trimethylamine N-oxide as a mediator of cardiovascular complications in chronic kidney disease. *Kidney Int* 2017;92:809-15.
34. Canyelles M, Borràs C, Rotllan N, Tondo M, Escolà-Gil JC, Blanco-Vaca F. Gut microbiota-derived TMAO: A causal factor promoting atherosclerotic cardiovascular disease? *Int J Mol Sci* 2023;24:1940.
35. Qi J, You T, Li J, Pan T, Xiang L, Han Y, et al. Circulating trimethylamine N-oxide and the risk of cardiovascular diseases: A systematic review and meta-analysis of 11 prospective cohort studies. *J Cell Mol Med* 2018;22:185-94.
36. Dai L, Massy ZA, Stenvinkel P, Chesnaye NC, Larabi IA, Alvarez JC, et al. The association between TMAO, CMPF, and clinical outcomes in advanced chronic kidney disease: Results from the European QUALity (EQUAL) study. *Am J Clin Nutr* 2022;116:1842-51.
37. Stubbs JR, Stedman MR, Liu S, Long J, Franchetti Y, West RE 3rd, et al. Trimethylamine N-oxide and cardiovascular outcomes in patients with ESKD receiving maintenance hemodialysis. *Clin J Am Soc Nephrol* 2019;14:261-7.
38. Chang D, Xu X, Yang Z, Ma T, Nie J, Dong J. Trimethylamine-N-oxide (TMAO) and clinical outcomes in patients with end-stage kidney disease receiving peritoneal dialysis. *Perit Dial Int* 2022;42:622-30.
39. Song Y, Wei H, Zhou Z, Wang H, Hang W, Wu J, et al. Gut microbiota-dependent phenylacetylglutamine in cardiovascular disease: Current knowledge and new insights. *Front Med* 2024;18:31-45.
40. Nemet I, Saha PP, Gupta N, Zhu W, Romano KA, Skye SM, et al. A cardiovascular disease-linked gut microbial metabolite acts via adrenergic receptors. *Cell* 2020;180:862-77.e22.
41. Shafi T, Meyer TW, Hostetter TH, Melamed ML, Parekh RS, Hwang S, et al. Free levels of selected organic solutes and cardiovascular morbidity

- and mortality in hemodialysis patients: Results from the retained organic solutes and clinical outcomes (ROSCO) investigators. *PLoS One* 2015;10:e0126048.
42. Poesen R, Claes K, Evenepoel P, de Loor H, Augustijns P, Kuypers D, et al. Microbiota-derived phenylacetylglutamine associates with overall mortality and cardiovascular disease in patients with CKD. *J Am Soc Nephrol* 2016;27:3479-87.
 43. Vogt I, Haffner D, Leifheit-Nestler M. FGF23 and phosphate-cardiovascular toxins in CKD. *Toxins (Basel)* 2019;11:647.
 44. Memmos E, Papagianni A. New insights into the role of FGF-23 and klotho in cardiovascular disease in chronic kidney disease patients. *Curr Vasc Pharmacol* 2021;19:55-62.
 45. Yamada S, Nakano T. Role of Chronic Kidney Disease (CKD)-mineral and bone disorder (MBD) in the pathogenesis of cardiovascular disease in CKD. *J Atheroscler Thromb* 2023;30:835-50.
 46. Filipka I, Winiarska A, Knysak M, Stompór T. Contribution of gut microbiota-derived uremic toxins to the cardiovascular system mineralization. *Toxins (Basel)* 2021;13:274.
 47. Pieniazek A, Bernasinska-Slomczewska J, Gwozdziński L. Uremic toxins and their relation with oxidative stress induced in patients with CKD. *Int J Mol Sci* 2021;22:6196.
 48. Townsend RR. Arterial stiffness in CKD: A review. *Am J Kidney Dis* 2019;73:240-7.
 49. Lioufas N, Hawley CM, Cameron JD, Toussaint ND. Chronic kidney disease and pulse wave velocity: A narrative review. *Int J Hypertens* 2019;2019:9189362.
 50. Tsai JP, Hsu BG. Arterial stiffness: A brief review. *Tzu Chi Med J* 2021;33:115-21.
 51. Vlachopoulos C, Xaplanteris P, Aboyans V, Brodmann M, Cifková R, Cosentino F, et al. The role of vascular biomarkers for primary and secondary prevention. A position paper from the European Society of Cardiology Working Group on peripheral circulation: Endorsed by the Association for Research into Arterial Structure and Physiology (ARTERY) society. *Atherosclerosis* 2015;241:507-32.
 52. Tomiyama H, Shiina K. State of the art review: Brachial-Ankle PWV. *J Atheroscler Thromb* 2020;27:621-36.
 53. Maruhashi T, Higashi Y. Current topic of vascular function in hypertension in 2023-2024. *Hypertens Res* 2024;47:3310-7.
 54. Saiki A, Ohira M, Yamaguchi T, Nagayama D, Shimizu N, Shirai K, et al. New horizons of arterial stiffness developed using cardio-ankle vascular index (CAVI). *J Atheroscler Thromb* 2020;27:732-48.
 55. Miyoshi T, Ito H. Arterial stiffness in health and disease: The role of cardio-ankle vascular index. *J Cardiol* 2021;78:493-501.
 56. Rossi M, Campbell KL, Johnson DW, Stanton T, Vesey DA, Coombes JS, et al. Protein-bound uremic toxins, inflammation and oxidative stress: A cross-sectional study in stage 3-4 chronic kidney disease. *Arch Med Res* 2014;45:309-17.
 57. Wang SC, Lai YH, Liu CH, Wang CH, Hsu BG, Tsai JP. Association between serum indoxyl sulfate levels with carotid-femoral pulse wave velocity in patients with chronic kidney disease. *Ren Fail* 2021;43:796-802.
 58. Chang YC, Lin YL, Lai YH, Wang CH, Hsu BG. Serum P-cresyl sulfate level is an independent marker of peripheral arterial stiffness as assessed using brachial-ankle pulse wave velocity in patients with non-dialysis chronic kidney disease stage 3 to 5. *Toxins (Basel)* 2022;14:287.
 59. Lai YH, Wang CH, Kuo CH, Lin YL, Tsai JP, Hsu BG. Serum P-cresyl sulfate is a predictor of central arterial stiffness in patients on maintenance hemodialysis. *Toxins (Basel)* 2019;12:10.
 60. Hsu BG, Wang CH, Lin YL, Lai YH, Tsai JP. Serum trimethylamine N-oxide level is associated with peripheral arterial stiffness in advanced non-dialysis chronic kidney disease patients. *Toxins (Basel)* 2022;14:526.
 61. Huang PY, Hsu BG, Lai YH, Wang CH, Tsai JP. Serum trimethylamine N-oxide level is positively associated with aortic stiffness measured by carotid-femoral pulse wave velocity in patients undergoing maintenance hemodialysis. *Toxins (Basel)* 2023;15:572.
 62. Huang PY, Lin YL, Chen YH, Hung SC, Liou HH, Tsai JP, et al. The association between serum trimethylamine N-oxide and arterial stiffness in chronic peritoneal dialysis patients: A cross-sectional study. *Toxins (Basel)* 2024;16:523.
 63. Yang HH, Chen YC, Ho CC, Hsu BG. Serum phenylacetylglutamine among potential risk factors for arterial stiffness measuring by carotid-femoral pulse wave velocity in patients with kidney transplantation. *Toxins (Basel)* 2024;16:111.
 64. Cunha RS, Santos AF, Barreto FC, Stinghen AE. How do uremic toxins affect the endothelium? *Toxins (Basel)* 2020;12:412.
 65. Harlacher E, Wollenhaupt J, Baaten CC, Noels H. Impact of uremic toxins on endothelial dysfunction in chronic kidney disease: A systematic review. *Int J Mol Sci* 2022;23:531.
 66. Rossi M, Campbell K, Johnson D, Stanton T, Pascoe E, Hawley C, et al. Uraemic toxins and cardiovascular disease across the chronic kidney disease spectrum: An observational study. *Nutr Metab Cardiovasc Dis* 2014;24:1035-42.
 67. Kamiński TW, Pawlak K, Karbowska M, Myśliwiec M, Pawlak D. Indoxyl sulfate – The uremic toxin linking hemostatic system disturbances with the prevalence of cardiovascular disease in patients with chronic kidney disease. *BMC Nephrol* 2017;18:35.
 68. Wang CH, Lai YH, Kuo CH, Lin YL, Tsai JP, Hsu BG. Association between serum indoxyl sulfate levels and endothelial function in non-dialysis chronic kidney disease. *Toxins (Basel)* 2019;11:589.
 69. Hobson S, Arefin S, Rahman A, Hernandez L, Ebert T, de Loor H, et al. Indoxyl sulphate retention is associated with microvascular endothelial dysfunction after kidney transplantation. *Int J Mol Sci* 2023;24:3640.
 70. Bethel M, Annex BH. Peripheral arterial disease: A small and large vessel problem. *Am Heart J Plus* 2023;28:100291.
 71. Wu CL, Targ DC. Targeting uremic toxins to prevent peripheral vascular complications in chronic kidney disease. *Toxins (Basel)* 2020;12:808.
 72. Kotov A, Blasche DA, Peters F, Pospiech P, Rother U, Stavroulakis K, et al. The impact of chronic kidney disease on mid-term outcomes after revascularisation of peripheral arterial occlusive disease: Results from a prospective cohort study. *J Clin Med* 2022;11:4750.
 73. Lin CJ, Pan CF, Liu HL, Chuang CK, Jayakumar T, Wang TJ, et al. The role of protein-bound uremic toxins on peripheral artery disease and vascular access failure in patients on hemodialysis. *Atherosclerosis* 2012;225:173-9.
 74. Wu CC, Hung SC, Kuo KL, Targ DC. Impact of indoxyl sulfate on progenitor cell-related neovascularization of peripheral arterial disease and post-angioplasty thrombosis of dialysis vascular access. *Toxins (Basel)* 2017;9:25.
 75. Lin TY, Chou HH, Huang HL, Hung SC. Indoxyl sulfate and incident peripheral artery disease in hemodialysis patients. *Toxins (Basel)* 2020;12:696.
 76. Chiu LT, Lin L, Lin HJ, Lai YH, Hsu BG. Positive correlation of serum indoxyl sulfate level with peripheral arterial disease in hemodialysis patients. *Vascular* 2022;30:928-33.
 77. Liu J, Zhong J, Yang H, Wang D, Zhang Y, Yang Y, et al. Biotic supplements in patients with chronic kidney disease: Meta-analysis of randomized controlled trials. *J Ren Nutr* 2022;32:10-21.
 78. Yu Z, Zhao J, Qin Y, Wang Y, Zhang Y, Sun S. Probiotics, prebiotics, and synbiotics improve uremic, inflammatory, and gastrointestinal symptoms in end-stage renal disease with dialysis: A network meta-analysis of randomized controlled trials. *Front Nutr* 2022;9:850425.
 79. Chen L, Shi J, Ma X, Shi D, Qu H. Effects of microbiota-driven therapy on circulating indoxyl sulfate and p-cresyl sulfate in patients with chronic kidney disease: A systematic review and meta-analysis of randomized controlled trials. *Adv Nutr* 2022;13:1267-78.

80. Nguyen TT, Kim HW, Kim W. Effects of probiotics, prebiotics, and synbiotics on uremic toxins, inflammation, and oxidative stress in hemodialysis patients: A systematic review and meta-analysis of randomized controlled trials. *J Clin Med* 2021;10:4456.
81. Zhang Y, Hu XY, Yang SY, Hu YC, Duan K. Effects of resistant starch supplementation on renal function and inflammatory markers in patients with chronic kidney disease: A meta-analysis of randomized controlled trials. *Ren Fail* 2024;46:2416609.
82. Takkavatakarn K, Puapatanakul P, Phannajit J, Sukkumme W, Chariyavilaskul P, Sitticharoenchai P, et al. Protein-bound uremic toxins lowering effect of sevelamer in pre-dialysis chronic kidney disease patients with hyperphosphatemia: A randomized controlled trial. *Toxins (Basel)* 2021;13:688.
83. Armani RG, Carvalho AB, Ramos CI, Hong V, Bortolotto LA, Cassiolato JL, et al. Effect of fructooligosaccharide on endothelial function in CKD patients: A randomized controlled trial. *Nephrol Dial Transplant* 2021;37:85-91.
84. Headley SA, Chapman DJ, Germain MJ, Evans EE, Madsen KL, Miele EM, et al. Effects of high amylose-resistant starch on gut microbiota and uremic toxin levels in patients with stage-G3a-G4 chronic kidney disease: A randomized trial. *J Ren Nutr* 2024;S1051-2276(24)00208-5.
85. Yu M, Kim YJ, Kang DH. Indoxyl sulfate-induced endothelial dysfunction in patients with chronic kidney disease via an induction of oxidative stress. *Clin J Am Soc Nephrol* 2011;6:30-9.
86. Ryu JH, Yu M, Lee S, Ryu DR, Kim SJ, Kang DH, et al. AST-120 improves microvascular endothelial dysfunction in end-stage renal disease patients receiving hemodialysis. *Yonsei Med J* 2016;57:942-9.
87. Su PY, Lee YH, Kuo LN, Chen YC, Chen C, Kang YN, et al. Efficacy of AST-120 for patients with chronic kidney disease: A network meta-analysis of randomized controlled trials. *Front Pharmacol* 2021;12:676345.
88. Chang G, Shih HM, Pan CF, Wu CJ, Lin CJ. Effect of low protein diet supplemented with ketoanalogs on endothelial function and protein-bound uremic toxins in patients with chronic kidney disease. *Biomedicines* 2023;11:1312.
89. Maheshwari V, Tao X, Thijssen S, Kotanko P. Removal of protein-bound uremic toxins using binding competitors in hemodialysis: A narrative review. *Toxins (Basel)* 2021;13:622.