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Contributory Role of Gut Microbiota and Their Metabolites Towards Cardiovascular Complications in Chronic Kidney Disease

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Abstract

The gut microbiome has recently emerged as a novel risk factor that impacts health and disease. Our gut microbiota can function as an endocrine organ through its unique ability to metabolize various dietary precursors, and can fuel the systemic inflammation observed in chronic disease. This is especially important in the setting of chronic kidney disease, where microbial metabolism can directly contribute to accumulation of circulating toxins that can then alter and shift the balance of microbiota composition and downstream functions. To study this process, advances in omics technologies are providing opportunities to understand not only the taxonomy but also functional diversity of our microbiome. We can also reliably quantify *en masse* a wide range of uremic byproducts of microbial metabolism. Herein, we examine the bidirectional relationship between the gut microbiome and the failing kidneys. We will describe potential approaches targeting gut microbiota for cardiovascular risk reduction in chronic kidney disease using an illustrative example of a novel gut-generated metabolite, trimethylamine *N*-oxide (TMAO).

Introduction

In the 1800s the English physician Richard Bright established a relationship between chronic kidney disease (CKD) and the clinical abnormalities brought about in the uremic state or the “residual syndrome” including cardiovascular disease (CVD)^{1, 2}. It has long been postulated that such increased CVD risk in CKD patients is partly due to a shared set of traditional risk factors such as diabetes, hypertension, albuminuria, dyslipidemia, and smoking³. However, such traditional risk factors do not entirely explain the accelerated progression of CVD in CKD patients. Since the large majority of morbidity and mortality of CKD remains CVD-related, elucidation of novel CVD risk factors may improve clinical outcomes of CKD patients.

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The contribution of specific gut microbiota generated metabolites towards uremia and poor outcomes have been documented in observational studies and associated with important clinical endpoints such as progression of renal failure, CVD events, and mortality^{4, 5}. Today, the perception of our microbiome has evolved from merely an inert set of microorganisms to a bona fide “endocrine organ.” Weighing in at approximately 2 kilograms, our intestinal microflora plays a vital role to maintain a symbiotic relationship with its host⁶.

Multiple efforts such as the Human Microbiome Project and MetaHIT are cataloging the human microbiome^{7, 8}. At birth, the gut microbiome is relatively sterile. However, continuous exposure to the environment results in subsequent colonization of trillions of bacteria ($> 10^{14}$)^{9, 10}. This composition of bacteria is now believed to be a sensitive factor that alters our risk for disease. In the setting of CKD, a comparison of the gut microbiota composition between those with end-stage renal disease (ESRD) and healthy individuals demonstrated the increased relative abundance of at least 190 bacterial operational taxonomic units (OTU)¹¹. The authors then extended their findings to a model of 5/6 nephrectomized rats which showed that a decrease in renal function also resulted in a distinct bacterial community¹¹. In addition, the mode of dialysis can also impact the gut microbiota composition¹². Based on these “environmental exposures,” our gut microbiome can generate products that are dependent on renal clearance that are either beneficial and/or detrimental to our health. In fact, production of uremic toxins may involve several different sources, ranging from the body itself (without intestinal processing), unmodified absorption of dietary products (such as advanced end glycation products), to metabolites that are produced in the intestine from precursors originating from microbial fermentation¹³ – all accumulating in the setting of impaired renal clearance.

How Uremic Solutes and Metabolites Impact the Microbiota

Dysbiosis, or imbalance of gut microbial composition, can stem from many processes including an altered diet as well as colonic compensation for increased uremic toxins. Specifically, uremic metabolites and solutes are produced with contributions at many steps including direct microbiota transformation, intestinal wall conjugation, liver conjugation or endogenous generation (Figure 1). As the glomerular filtration and compensatory tubular secretion become impaired in CKD and tubular injury, increased nitrogen compounds such as urea and uric acid may instead excreted via the intestines. As an example, increased urea secretion through the gastrointestinal tract is converted to ammonium which then leads to elevated intraluminal pH as well as mucosal damage¹⁴. This influx of nitrogen-rich compounds, along with the damage incurred by its metabolic byproducts, can lead to further gut dysbiosis. In the setting of elevated uremic toxins, bacterial species that contain enzymes capable of metabolizing nitrogen compounds such as p-cresol and indole, urea, and uric acid as sources for fermentation will out-proliferate bacteria such as *Lactobacillae* or groups that utilize dietary fiber to convert into short chain fatty acids (SCFA)¹⁵. These functional changes have been reported by Wong et al. in which 19 families of bacteria containing urease, uricase, and indole or p-cresol-forming enzymes were identified predominantly in the setting of ESRD¹⁵, while there were decreased bacterial families possessing SCFA butyrate-forming enzymes. Many other external factors can also influence gut microbial composition. For example, patients with CKD maintains a difficult balance between

adequate nutrition to promote symbiosis while avoiding potassium and phosphorus overload¹⁶. Physiologic factors such as gut edema and reduced intestinal transit will influence the intestinal microflora. Patients with CKD often require chronic medications including phosphate binding agents, iron supplements as well as antibiotics that can directly or indirectly influence gut microbial composition.

The Microbiota as a Driver of Persistent Inflammation in CKD

In a healthy individual, the mucosal lining of the gut epithelium consists of the mucus layer as well as protective factors such as defensins and lectins that can create a barrier between the epithelium and immune system from contact with the microbiota^{17, 18}. However, in autopsy studies of hemodialysis patients from the 1980s, chronic inflammation was noted throughout the gastrointestinal tract beginning from the esophagus and ending at the colon¹⁹. The source of inflammation was attributed to factors such as the dialysis interface, subclinical infection, iron administration or pre-existing cardiac dysfunction²⁰. Further investigation of intestinal permeability in rats showed that molecular markers such as polyethylene glycol of varying molecular weight were detectable in rats with CKD²¹. Structurally, the intestinal epithelium consists of claudins, occludin, E-cadherin and zona occludens which help protect the epithelial junctions²². Studies targeting these epithelial factors confirmed a depletion of the tight junction proteins such as claudins, occludin-1, and zona occludens-1²³. This increase in permeability is hypothesized to allow for a “leaky gut” and thus contribute to chronic endotoxemia as well as the increased passage of uremic solutes from the gut into the body²⁴. Sequencing of the collecting lymph nodes in the mesentery demonstrated that that colonic bacteria could also be detected²⁵. The presence of microbes that pass through the gut barrier can further induce several families of pattern recognition receptors including the NLRP3 (nucleotide-binding oligomerization domain [NOD]-like receptor family, pyrin domain containing 3) inflammasome, toll-like receptors, and NOD-like receptors²⁶. Once tight junction breakdown occurs through multiple means, experimental evidence shows that increased levels of urea during decreased clearance results in compensatory excretion through the gut²⁷. Accumulation of urea in the gut lumen results in a different nutritional source that is utilized by gut bacteria that contain urease. This result in the production of ammonia which is then hydrolyzed to ammonium hydroxide, which can injure the gut epithelial barrier^{14, 27}. This injury results in recruitment of inflammatory cells such as leukocytes which then feeds forward into increased levels of cytokines that stimulate endocytosis of the tight junction proteins.

Direct evidence for intestinal breakdown and subsequent bacterial translocation as a source of inflammation in CKD was shown by Anders et al. using a Col4a3-knockout mice to simulate CKD²⁸. In their model, a co-housing strategy was used to ensure that cage effects did not bias the results. The uremic mice developed increased inflammation with increased levels of pentraxin-2, interleukin(IL)-6, and T cells in the spleen. However, IL-1 β , another cytokine well described to influence gut permeability was statistically unchanged. Bacterial sequencing also showed demonstrable alterations in the gut flora of the Col4a3-knockout mice. To then confirm a microbiota mediated inflammatory effect, the authors used antibiotics in the uremic mice and demonstrated decreased inflammatory factors, decreased

activated T cells, and a recovery of regulatory T cells. More importantly, renal function was not changed, which rules out antibiotic effects on the kidney itself.

Targeting inflammatory mediators as a method to reduce CVD has recently been shown in humans in the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS)²⁹. Blocking IL-1 β led to reduced CVD events but those with heart failure, nephropathy or estimated glomerular filtration rate <30 ml/min/1.73m² were not included in this study. In CKD, another avenue of exploration is the nuclear transcription factor Nrf2, which has been investigated for its anti-oxidant and anti-inflammatory roles³⁰. Lau et al. have shown that inflammation suppresses Nrf2 expression, but treatment with Nrf2 agonists can result in improved expression of tight junction proteins as well as improved inflammation³¹. However, a phase 3 trial using a Nrf2 agonist, bardoxolone methyl, to delay ESRD progression surprisingly resulted in increased CVD adverse events³². Hence, further investigations of strategies that target the instigators or mediators of inflammation in CKD are therefore warranted, which prompted the growing interests in gut microbiota.

Evidence for Gut Microbiota Derived Uremic Toxins

In recent years, rapid expansion of “-omics” technology has enabled “high-throughput” discoveries at every level of biology, which is in stark contrast of a century ago when phlebotomy and subsequent analysis of urea in the blood and urine was state of the art¹. The contribution of gut microbiota towards the generation of uremic toxins has been investigated over half a century when gnotobiotic anephric rats were observed to have longer survival compared to their germ-inhabited counterparts³³. As early as in the 1970s, patients with CKD were found to have significantly altered microbiota³⁴, and that alteration of the intestinal bacterial flora with antibiotics markedly reduced serum dimethylamine and trimethylamine concentrations and modified mental status symptoms³⁵. Furthermore, removal of the microbiota via antibiotic use also resulted in both the fecal and urinary excretion of phenolic and aromatic bacterial metabolism products in a pig model³⁶. These seminal observations established the association between gut microbiota-derived uremic toxins and CKD disease progression.

Today, integration of metabolomics, proteomics, and microbiomics can accelerate the discovery of novel retained uremic solutes. For example, combining proteomics and metabolomics will allow the identification and quantification of proteins, peptides as well as small metabolites under 1,000 Da. These products represent the phenotypes of both the host and microbial metabolism. We can then assimilate the findings of the microbiota through both the taxonomy via 16s sequencing as well as microbial community dynamics by metagenomic sequencing. Indeed, accumulation of multiple colonic-derived metabolites has been identified through untargeted metabolomics in biological samples from dialysis patients, in which specific metabolites known to be generated by colonic microbiota were noticeably absent after colectomy while being >20-fold higher without colectomy³⁷. Moreover, these metabolites were able to distinguish patients with and without colectomy after hierarchical clustering. This survey allowed identification and confirmation of multiple colonic-derived metabolites, even though the authors were unable to clarify the identity of many of these metabolites. A similar approach utilized untargeted metabolomics in the

plasma produced by intestinal microbiota of rats with normal kidney function, among them ~10% were considerably different between the gnotobiotic and conventionally-raised rats³⁸. Amongst the identified discriminators, the authors identified nine indole and phenyl derivatives, with many overlapping with previous CKD studies from Aranov et al. along with seven other solutes that were elevated. Of note, these metabolites were also noted to be substrates of common xenobiotic transporters such as organic anion transporters. Applications of these retention solutes are further demonstrated in a variety of studies using multi-marker methodologies to expand the prognostic capabilities in CKD. For example, in the Framingham cohort, a multi-marker panel of such metabolites could predict the development of CKD after adjustments for risk factors such as kidney function, demographics, comorbidities, and proteinuria at baseline³⁹. From their screen of over 200 metabolites, those in the tryptophan, choline, and purine metabolic pathways were highly associated in the CKD population. They further quantified which metabolites had a higher secretion potential by measuring the venous to arterial metabolite ratios in 9 patients as a surrogate for renal tubular involvement. Another metabolomics screen in stage 3-4 CKD patients revealed 14 elevated metabolites in those with disease. This screening process identified several known uremic solutes including p-cresyl sulfate (pCS). Moreover, two novel metabolites, dimethyl sulfone, and 2-hydroxyisobutyric acid were also found⁴⁰.

An updated review of uremic retention solutes by the European Uremic Toxin Work Group has now listed over 150 different compounds⁴¹. Given the relative ease for novel small molecular discovery to associate with the CKD phenotype, it is also imperative for the pathophysiologic mechanisms of these novel uremic retention solutes to be investigated, especially in relation to gut microbiota metabolism. Here, we take a closer look at the beneficial bacterial fermentation product SCFA, the well-studied uremic solutes indoxyl sulfate (IS) and p-CS), and the more recently discovered trimethylamine N-oxide (TMAO). A recent review from Ramezani et al. provides primers on additional uremic solutes⁴².

Short Chain Fatty Acids

On the beneficial spectrum of microbiota generate metabolites, distal gut anaerobic fermentation of undigested nutrients such as resistant starches, dietary fiber, various polysaccharides produce organic fatty acids containing one to six carbons called short-chain fatty acids (SCFAs). The majority of SCFAs consist of acetate, butyrate, and propionate with a distribution of 60% acetate and equal 20% amounts of butyrate and propionate^{43, 44}. The colonic epithelium efficiently absorbs SCFAs through both passive and active mechanisms⁴⁵. The variety of host functions affected by SCFAs has been expanding. The most abundant SCFA readily detectable in the blood, acetate, is a source of energy for peripheral cells⁴⁶. Similarly, butyrate provides energy for the maintenance of the ceco-colonic epithelium while propionate is a source for gluconeogenesis in the liver. Together, these SCFA are estimated to provide between 5 to 10 percent of the energy source in healthy individuals⁴³.

More than just a source of nutrition, recent studies have suggested that SCFAs also play important roles in processes, such as regulation of blood pressure and modulation of systemic inflammation. Increased fruit and vegetable consumption has also been shown to

result in increased acetate production. This increase in acetate has been demonstrated to reduce blood pressure while preventing renal and cardiac fibrosis⁴⁷. Recently, several novel receptors such as G-protein coupled receptors 41 and 43 (Gpr41, Gpr43), have been identified to be targets of SCFA⁴⁸. However, the methods by which SCFA can alter Gpr43 has been shown to counteract increases in renin secretion by olfactory receptor 78 (olfr78) in the kidney⁴⁹. SCFAs plays a major role in inflammation and mediating intestinal tight junction integrity⁵⁰. SCFAs have been shown to prevent inflammation through increased anti-inflammatory cytokine IL-10 and decreased proinflammatory IL-12 and tumor necrosis factor alpha (TNF- α) secretion⁵⁰⁻⁵². While gnotobiotic mice are more susceptible to ischemia-reperfusion kidney injury, treatment with SCFAs can protect mice through decreased inflammation, decreased apoptosis, and increased autophagy⁵³.

Indoxyl Sulfate and P-Cresyl Sulfate, Colon Derived Solutes

Indoxyl sulfate and its related indole compounds are produced by sulfation in the liver after degradation of tryptophan by bacterial tryptophanase. Similarly, pCS is a major circulating metabolite of p-cresol conjugation in the intestinal wall⁵⁴. p-Cresol is a product of colonic bacterial fermentation from phenylalanine and tyrosine. Both IS, and pCS are protein-bound and are not readily filtered by the kidney or removed by hemodialysis⁵. Instead, it is dependent upon secretion by organic anion transporters and accumulates during CKD progression. Niwa described this “protein metabolite” hypothesis in 1997, and because of this physiology, these metabolites have been the focus of studies in uremia⁵⁵.

Many clinical studies have examined the association between IS and pCS with both CKD progression and their potential contribution to CVD. Circulating levels of IS and PCS have been associated with the severity of CKD. Elevated serum IS has been directly associated with aortic calcification, arterial stiffness represented by altered pulse wave velocity, peripheral vascular disease, and mortality⁵⁶. Similarly, pCS levels correlated with impaired renal function and increased CVD events⁵⁷. Furthermore, a prospective study of 268 patients with CKD found associations for both IS and pCS with predicated loss of kidney function⁵⁸, and a meta-analysis has found associations for both PCS and IS with mortality, although only pCS (not IS) was associated with CVD mortality⁵⁹.

A wide array of mechanisms for which IS and PCS can contribute to both CKD and CVD has been explored and summarized in Table 1. Early investigations showed that IS administration to rats was capable of stimulating monocyte infiltration with the production of factors related to tubulointerstitial fibrosis such as transforming growth factor beta (TGF- β) or tissue inhibitor of metalloproteinases (TIMPs)⁶⁰. IS has found be mechanistically related to multiple CVD phenotypes⁶¹. Similar to IS, direct administration of PCS promotes renal tubular damage in 5/6-nephrectomized rats that were attributed to alterations in NADPH oxidase activity, reactive oxygen species (ROS) production, and induction of inflammatory cytokines⁶². As previously mentioned, pCS and IS are protein bound and not easily removed via hemodialysis. Therefore, it is important to assess their effects as a measure of free concentration and not of total solute concentration. A systematic review analyzed 27 studies in which free concentrations of PCS and IS were studied in cell types such as the endothelium, hepatocyte, tubular cells, and cardiac tissue⁵. This rigorous review

with stringent standards confirms the toxicity of IS and pCS and support their roles in vascular and renal disease progression.

Trimethylamine N-oxide, a Meta-organismal-Derived Metabolite

Trimethylamine N-oxide is a product of bacterial metabolism of dietary precursors that contain the trimethylamine (TMA) moiety such as phosphatidylcholine and carnitine^{63, 64}. TMA generated by bacterial lyases are efficiently absorbed by the intestine and subsequently converted to TMAO by liver flavin-containing monooxygenase 3 (FMO3) in the human host⁶⁵. Classically, TMAO has been studied for its activity as an organic osmolyte and helps counterbalance the extreme salinity and osmotic pressures which deep-sea fish encounter⁶⁶. This molecule has also been studied for its molecular chaperone properties⁶⁷, and has long been thought to be a nitrogenous waste metabolite that is largely excreted by the kidneys.

Over the past years, TMAO has emerged as a novel contributor to the development of atherosclerosis, and the scope of TMAO research has rapidly expanded towards other diseases such as CKD and heart failure^{4, 68}. Wang et al. first identified TMAO through an untargeted metabolomics screen of patients at high risk for cardiovascular disease, and confirmed these findings in a large prospective cohort in which elevated fasting levels of TMAO portend poorer long-term major adverse cardiac events^{69, 70}. In ApoE null mice, direct TMAO feeding was observed to cause atherosclerosis while antibiotics resulted in decreased atherosclerotic plaques. These findings established a direct microbiota-dependent link between dietary nutrients, gut microbiota, and CVD pathogenesis, and are further confirmed by cecal as well as microbial transplant studies that demonstrated transmissibility of microbiota-specific effects that satisfied Koch's postulate⁷¹. Specifically in CKD, fecal transplant experiments demonstrated that transfer of microbiota from CKD patients to antibiotic-treated mice produced elevated plasma TMAO with choline-feeding and alterations in the TMAO metabolism functional profile⁷². Recently, a targeted competitive inhibitor of TMA lyase was able to reduce both circulating TMAO levels and atherosclerosis progression⁷³. Recently, simultaneous publications of meta-analysis from three different groups had largely confirmed that elevated TMAO levels is associated with increased CVD risk and mortality⁷⁴⁻⁷⁶.

There have been major efforts to unravel the mechanistic underpinnings of adverse effects of TMAO. Original studies implicated TMAO in alterations of cholesterol metabolism with upregulation of scavenger receptors of macrophages^{64, 70}. Several recent studies have expanded the effects of TMAO exposure on promoting platelet hyperresponsiveness, as well as robust inflammatory responses via the NF- κ B pathway and inflammasome activation from endothelial and vascular smooth muscle cells⁷⁷⁻⁷⁹. Interestingly, the TMAO precursor TMA is known to interact with the olfactory receptor Trace Amine-Associated Receptor 5 (TAAR5), a G-protein coupled receptor, even though TAAR5 does not recognize TMAO⁸⁰. Further investigations are warrant in identifying a potential receptor-mediated mechanism for TMAO that would greatly advance the field.

TMAO is a predominant kidney-cleared metabolite. In the context of CKD, reduced renal clearance unsurprisingly results in accumulation of TMAO, while renal transplantation

results in dramatic reduction in plasma TMAO levels^{81, 82}. Several reports have investigated the renal impact and prognostic value of TMAO in the CKD population (Table 2). Tang et al. first showed that elevated TMAO in patients with reduced eGFR was prognostic for poor outcomes⁴. The authors also demonstrated that choline and TMAO-fed mice directly promoted renal fibrosis and was associated with increased phospho-Smad3 (a TGF β -signaling pathway), as well as markers of renal dysfunction such as cystatin C and kidney injury marker-1⁴. Subsequent studies have observed that in those with estimated glomerular filtration rate <60 ml/min/1.73m² TMAO levels were associated with atherosclerotic burden⁸². There also appears to be racial differences in the prognostic potential of TMAO with white patients at greater risk than black patients, which interestingly falls in line with the dialysis vintage disparity between black and white patients^{83, 84}. A recent study has even indicated that TMAO may confer stronger prognostic value in patients with any degree of renal dysfunction⁸⁵.

Despite the numerous clinical associations reported for TMAO in CKD patients, mechanisms for TMAO excretion has been scant to date. Recently, a specific renal transporter for TMAO has been suggested by several in vitro studies^{86, 87}. These studies identified organic cation transporter 2 (OCT2), a well-studied xenobiotic transporter as being involved in TMAO excretion. In kinetic experiments, the K_m of OCT2 for TMAO has been reported to range from 7mM to 72mM. Unfortunately, given that the average concentration of TMAO in the general population is only in the micromolar range (a 1,000 $\times$ fold difference), the possibility of OCT2 being a physiologically important transporter is less plausible⁶⁹. Although OCT1/2 knockout mice exhibited slightly elevated TMAO levels, no OCT2 polymorphisms were associated with changes in plasma TMAO^{86, 87}. Furthermore, introduction of TMAO at the levels observed in uremia did not alter the transport of metformin, a prominent OCT2 substrate⁸⁸.

From clinical observations, dialytic clearance data used to calculate the differences in TMAO and creatinine clearance found that those with CKD appeared to have reduced TMAO clearance capacities⁸⁹. Moreover, in healthy individuals with normal renal function, they appeared to have increased TMAO clearances above that of creatinine, suggesting that the kidneys actively secrete TMAO. In another experiment, TMAO was identified as one of 16 metabolites that were associated with the development of CKD in prospective follow-up³⁹. While venous-arterial concentration ratio of choline was less than creatinine, that of TMAO appeared to be similar to creatinine in a series of patients with CKD, suggesting that those with reduced kidney function also appeared to have a loss of TMAO secretory function.

Therapeutic Options Targeting Gut Microbiota

Multiple approaches are taken in therapy which ranges from direct modification of diet, prebiotics, probiotics, synbiotics, toxin-absorbing products, and finally, novel advances in the field suggest the potential for targeted interventions through bacterial enzyme inhibition. Here, we do not focus on specific microbial species, as significant discrepancies are reported in the literature. Even bacteria amongst the same taxa may contain different functional

capacities. However, expansion of new technology such as metagenomics will be able to better distinguish this varying functional capacity of our microbiota.

Dietary Modification

Dietary exposure may be one of the biggest contributors towards balancing the intestinal microbiota composition. Vegetarian diets in normal populations have decreased concentrations and generating capacities of protein-bound uremic solutes such as pCS and IS^{44, 90}. Though a low-protein diet is often recommended in dietary guidelines for CKD patients, large studies such as the Modification of Diet in Renal Disease has found little benefit in preventing CKD progression^{91, 92}. In a study involving 40 patients with moderate-to-advanced CKD, a lower ratio of dietary fiber to protein was associated with higher blood levels of IS and pCS⁹³. Although interventions in dietary changes such as excluding dietary toxin sources have been attempted with promising results^{94, 95}, it is a major lifestyle change and can be very difficult to adhere to. It is important to note that “heart healthy” diets are often high in dietary fiber that may lead to the production of protective SCFAs largely come from sources such as vegetables and fruits^{43, 47}. Unfortunately, this type of diet also contains high levels of potassium and phosphorus, which when faced with poor clearance in CKD patients, can be associated with increased mortality risk⁹⁶. This is an area that would benefit from more rigorous and scalable interventions even with unconventional diets and taking into account of gut microbial compositions^{96, 97}.

Prebiotics, Probiotics, and Synbiotics

Prebiotics are indigestible compounds which can improve the microbiota composition and subsequently, the functional capacity⁹⁸. Probiotics then are living organisms that can be consumed⁹⁹. Finally, synbiotics contain a mixture of these two products. The findings from these studies have been positive but inconsistent, and often only a partial set of “important” uremic toxins were shown to be decreased. Administration of prebiotics such as inulin or resistant starch was shown to decrease pCS and IS, respectively^{100, 101}, while decrease in IS was observed when given a lactic acid bacteria preparation IS¹⁰² or *Bifidobacterium* in a gastric acid-resistant pill¹⁰². Examples of synbiotics include work from Nakabayashi et al. where a preparation combining *Lactobacillus*, *Bifidobacterium*, and galacto-oligosaccharides resulted in decreased pCS¹⁰³. In the SYNERGY trial, CKD patients received 15g/day of a mixture of fermentable dietary fibers and probiotic strains from the *Bifidobacterium*, *Lactobacillus*, and *Streptococcus* genera also observed reduction in pCS but not IS¹⁰⁴.

Absorption Therapy

A prominent example of adsorption therapy is the oral activated charcoal adsorbent, AST-120. This polymer has been shown in preclinical studies to restore colonic tight junction proteins as well as decreasing plasma endotoxin and inflammatory markers¹⁰⁵. These therapies had strong preclinical data that supported its ability to reduce IS and pCS levels. However in randomized controlled trials, AST-120 did not yield beneficial outcomes, largely due to being underpowered by lower event rates¹⁰⁶.

Over the past years, the number of known uremic solutes has steadily increased to over 150⁴¹. It is clear that no single factor can be held responsible for the progression of these complex trait diseases, and different metabolites may have different characteristics. As an example, TMAO has demonstrated unique traits which differ from that of IS and pCS which can help to serve as another model microbial metabolite in the development of biomarkers for diagnostic and prognostic purposes. It is not protein bound like IS and pCS and yet is still poorly removed during hemodialysis⁸⁹. This occurs in part due to decreased volume of distribution as well as the more efficient excretion of TMAO by the kidney compared to the dialytic standard urea. Observations from Aranov et al. have shown that colectomy does not alter levels of monomethyl- and dimethylarginine levels compared to that of IS and pCS³⁷. Curiously, the AST-120 studies in nephrectomized rats also reveal significant increases in TMAO levels despite decreases in IS and pCS¹⁰⁷. This evidence illustrates the need for future studies towards uremic toxin removal to be improved through experimental rigor and a systematic approach.

Targeted Inhibition of Microbial Enzymes

Along with the ideas of absorbing excess toxins, targeted reduction of uremic toxin production has been demonstrated for TMAO with robust preclinical data. The small molecule mimetic of choline, 3,3-dimethyl-1-butanol (DMB), is a potent antagonist against a wide array of TMA lyases without affecting their survival⁷³. Wang et al. demonstrated that DMB feeding along with TMAO showed in non-lethal inhibition of TMA generation. Specifically, choline-fed ApoE^{-/-} mice treated with DMB demonstrated decreased plasma TMAO levels along with attenuated atherosclerosis development compared to no DMB treatment⁷³. This finding extends previous work with antibiotics for targeted elimination of TMAO generation, and may have a lower likelihood of developing “resistance” than antibiotics. Interestingly, DMB is also found naturally in foods such as extra-virgin olive oil, which is a prominent component of the Mediterranean diet.

Meanwhile, studies have identified various compounds being able to modify TMAO levels, including aspirin¹⁰⁸, resveratrol¹⁰⁹, and meldonium (an analog of γ -butyrobetaine)¹¹⁰. The exact mechanisms are largely unknown. Additional validation studies will be important to assess the potential utility of these treatments.

Conclusion

Gut microbiota is now recognized as an important contributor to health that is easily perturbed not only by host factors but also by lifestyle and environmental changes. Gut microbiota can stimulate the immune system to cause inflammation, and have profound impact on the host by metabolizing dietary precursors and generate metabolites with both beneficial and toxic paracrine and endocrine effects. Large scale – omics technology will now enable us to better quantify the litany of circulating metabolites to identify novel patterns to diagnose, predict, and treat disease. However, systematic approaches are necessary to study this subject and beyond just knowing that these microbial metabolites are present, but also understanding their mechanistic contributions. Rigorous methodology along with the state-of-the art technologies will pave way for novel therapeutic discoveries

for CKD patients to prevent CVD as we continue to explore and develop our understanding in this uncharted territory.

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Figure 1.

Dietary precursors are modified by the microbiota to generate an array of uremic toxins through multiple mechanisms at the level of the microbiota, intestinal wall, and liver. Influx of uremic toxins results in microbiota imbalance along with bacterial translocation and inflammation. These factors contribute to the multitude of systemic effects covered in this review.

Figure 2.

Overview of TMAO metabolism. Dietary precursors such as red meat, fish, and eggs contain compounds that contain the TMA moiety which is released as TMA, absorbed into circulation and converted to TMAO in the liver. Circulating TMAO has been implicated in processes such as atherosclerosis, platelet activation as well as vascular inflammation. These processes contribute to the development of renal dysfunction. Destruction of tubular secretion mechanisms result in the accumulation of TMAO in the disease setting and contribute to a feed-forward cycle. *Modified from Tomlinson et al. 2017.*

Table 1

Summary of Mechanistic Studies on Indoxyl Sulfate and P-Cresyl Sulfate Effect on the Heart and Kidney

Author	Cell/Organ System	Uremic Toxin	Main Observed Effect	Molecular Mediator Implicated
Dou 2007 ¹¹¹	Endothelium	IS	Induction ROS, inhibition glutathione	NADPH Oxidase ↑
Ito 2010 ¹¹²	Endothelium	IS	Increased endothelial/leukocyte interaction	NADPH oxidase; E-selectin ↑
Lekawanvijit 2010 ¹¹³	Cardiac Fibroblast	IS	Cardiac fibrosis and inflammation	Cytokines and Mitogen-activated protein kinase: MEK 1/2; p38; Nuclear Factor-kB ↑
Bolati 2011	Tubular Cell	IS	Increased epithelial to mesenchymal transition	E-cadherin ↓
Sun 2012 ¹¹⁴	Proximal Tubular Cell	IS, PCS	Increased cytokine and inflammatory gene expression	α-Smooth muscle actin; Smad2/3 and Smad4 ↓
Sun 2012 ¹¹⁵	Proximal Tubular Cell	IS, PCS	Increased Klotho gene methylation and renal fibrosis	Klotho ↓
Koppe 2013 ¹¹⁶	Myotube, Myocyte	PCS	Increased insulin resistance, increased lipolysis, decreased lipogenesis	Phosphoinositide 3-kinase; Insulin receptor substrate-1; Extracellular-regulated kinase 1/2 ↓
Chitalia 2013 ¹¹⁷	Smooth Muscle Cells	IS	Increased tissue factor generation	Tissue factor ↑
Yisireyili 2013 ¹¹⁸	Cardiac Tissue	IS	Increased cardiac hypertrophy, fibrosis, and oxidative stress	Nuclear Factor (erythroid-derived 2) - like 2; Heme-oxygenase-1 ¹¹⁹ ↓ Transforming Growth Factor-β ↑
Bolati 2013 ¹²⁰	Tubular Cell	IS	Increased oxidative stress	Nuclear Factor (erythroid-derived 2) - like 2; Heme-oxygenase-1 ↓
Sun 2014 ¹²¹	Proximal tubular cell	IS, PCS	EGF receptor activation	EGF receptor downstream signaling ↑
Han 2015 ¹²²	Cardiac tissue	PCS	Production of reactive oxygen species	NADPH oxidase ↑
Hung 2016 ¹²³	Endothelial progenitor cells	IS	Impaired neovascularization	HIF-1α and interleukin-10/STAT3 ↓
Yang 2017 ¹²⁴	Platelet	IS	Induce platelet hyperreactivity	ROS/p38MAPK ↑

Selected studies include those rated as “Strong” in review from Vanholder et al. 2014 along with selected publications since 2014.

Note – P-cresol, the precursor to PCS was originally thought to be the retention solute due to an unforeseen preparation artifact¹²⁵. It was only the early 2000s when PCS was identified and its biologic toxicity sought out.

Abbreviations: IS – Indoxyl Sulfate; PCS – P-Cresyl Sulfate

Summary of Clinical Investigations Between TMAO and Cardiovascular Related Outcomes in Various Patient Cohorts

Study Cohort	Author	Subjects	Study Population Criteria	Endpoint of Interest	Results
Chronic Kidney Disease	Tang et al. ⁴	521	Stable subjects with CKD (estimated glomerular filtration rate, <60 mL/min per 1.73 m ²)	All-cause mortality over 5 years of highest quartile TMAO	HR 1.93, CI 1.13–3.29, P<0.05
	Stubbs et al. ⁸²	220	Individuals with CKD defined as eGFR<60 mL/min per 1.73 m ²	Atherosclerotic burden by modified Gensini Score; 4 year mortality per 10 µM TMAO	TMAO to Gensini score correlation r=0.17, P=0.02; HR 1.26, CI 1.13 – 1.40, P<0.001
	Shafit et al. ⁸⁴	1,232	Mixed ethnicity cohort (35% white, 65% black) with available serum from the HEMO study group ¹²⁶ randomized clinical trial comparing standard or high dialyzer urea clearance.	Composite of death by coronary event, arrhythmia, sudden cardiac death, congestive heart failure, or cerebrovascular; any-cause death over years of highest quintile TMAO comparing white vs black patients	HR, 3.89; 95% CI, 2.47 to 6.13; P<0.001; HR 1.92, CI 1.28–2.86, P=0.001
	Kim et al. ¹²⁷	2,529	CKD patients with Stage 3b and 4 CKD, under nephrology care, in urban and rural centers across Canada.	Time to myocardial infarction, unstable angina, ischemic stroke, coronary revascularization, new onset coronary heart disease, amputation, peripheral artery bypass, and gangrene over 3 years per ln TMAO	HR 1.23, CI 1.06–1.42, P = 0.0059
	Missailidis et al. ¹²⁸	179	CKD 3–4 and CKD 5 patients classified according to eGFR	All-cause mortality over 5 years of combined mid and high tertile TMAO	HR 4.32, CI 1.32–14.2, P = 0.016
Meta-analysis	Kaysen et al. ¹²⁹	Cardiovascular mortality = 152 for; All-cause mortality = 235	Adults with end-stage renal disease who initiated hemodialysis or peritoneal dialysis	Time to death; Time to cardiovascular death or hospitalization per unit log TMAO	HR 0.84, CI 0.65–1.09 P = 0.19; HR 0.88, CI 0.57–1.35 P = 0.55
	RobinsonCohen et al. ¹³⁰	339	Adults with eGFR 90 mL/min/1.73m ² or a urinary protein to creatinine ratio of > 30 mg/g	FMO3 variants at amino acid 158 and association with and mortality after 3.3 years median follow-up	E/K heterozygous: HR 1.97, CI 0.85–4.60; K/K Homozygous: 2.22-fold, CI 0.89–5.48; additive model P = 0.04
	Gruppen et al. ⁸⁵	5,469	Individuals age 28 to 75 from the Prevention of Renal and Vascular End-Stage Disease (PREVEND) study with measured TMAO values stratified by renal function	Highest vs lowest quartile TMAO all-cause mortality in subjects with eGFR <90 mL/min/1.73 m ²	HR 1.18, CI 1.02–1.36 P = 0.023
	Heianza et al. ⁷⁶	19,256	PubMed and Embase, 16 publications	Associations between TMAO with the development of MACE or death	RR1.62; CI 1.45–1.80; I ² =23.5%
	Schiattarella et al. ⁷⁴	15,662; 13,944	MEDLINE, ISI Web of Science and SCOPUS, 17 publications	Associations between TMAO plasma levels with all-cause mortality; MACCE; dose response	HR 1.91, CI 1.40–2.61, P < 0.0001, I ² = 94%; HR 1.67, CI 1.33–2.11, P < 0.00001, I ² = 46%;

Study Cohort	Author	Subjects	Study Population Criteria	Endpoint of Interest	Results
	Qi et al. ⁷⁵	8,139; 7,716	PubMed and Embase, 11 publications	Association between TMAO plasma levels with cardiovascular events; all-cause mortality	RR per each 10 µmol/L increment of TMAO 1.07, CI 1.04–1.11, P < 0.0001 HR 1.23, CI: 1.07–1.42, I ² = 31.4%; HR 1.55, CI: 1.19–2.02, I ² = 80.8%

Updated from Li et al. 2017¹³¹

Abbreviations: CI – 95% Confidence Interval; CKD – Chronic Kidney Disease; eGFR – estimated Glomerular Filtration Rate; HR - Hazard Ratio; MACE – Major Adverse Cardiovascular Events; MACCE - Major Adverse Cardiac and Cerebrovascular Events; RR – Risk Ratio