Minireview

The role of chronic kidney disease-associated dysbiosis in cardiovascular disease

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Impact statement

Negative alterations, or dysbiosis, in the intestinal microbial community balance in response to chronic kidney disease is emerging as a substantial and important factor in inducing and exacerbating multiple comorbid conditions. Patients with renal insufficiency experience a substantial increase in cardiovascular risk, and recent evidence is shedding light on the close interaction between microbiome dysbiosis and increased cardiovascular events in this population. Previous association and recent causality studies utilizing experimental animal models have enriched our understanding and confirmed the impact of microbial community imbalance on cardiac health in both the general population and in patients with renal impairment.

Abstract

Survival outcomes of patients with end stage renal disease are worse than those of many metastatic cancers. Kidney disease patients are often inflicted with higher rates of cardiovascular disease, in which nearly half of the mortalities are attributed to adverse cardiovascular events. Of the multifarious reasons for this detrimental impact, dysbiosis in the intestinal microbiome is surfacing as a potential participant. This is likely due to the numerous metabolic and inflammatory shifts found in chronic kidney disease, as well as environmental changes within the intestinal lumen. Studies are beginning to link microbiota alterations mediated by chronic kidney disease to negative cardiovascular outcomes. Here, recent findings connecting dysbiosis in chronic kidney disease and various cardiovascular insults are reviewed.

Keywords: Chronic kidney disease, dysbiosis, cardiovascular disease, microbiome, uremic toxins, hypertension

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Introduction

The human gastrointestinal tract houses upwards of 10^{14} micro-organisms that are involved in complex interactions with the host and the outside environment.[1,2] The organismal microenvironment is amenable to changes in response to different factors.[3–8] As demonstrated by the human microbiome project (HMP) and the European Metagenomics of the Human Intestinal Tract (MetaHIT), the healthy microbiome can vary from one individual to another,[9] but remains relatively stable over time.[10–12] The microbiome-host relationship carries a spectrum of beneficial roles in energy utilization, storage and nutrition,[2] immune system regulation and adaptation,[13] intestinal integrity,[14–16] handling and processing complex carbohydrates,[17,18] and the production and absorption of different vitamins.[3,18–22] It also contributes to amino acid homeostasis such as synthesizing lysine and threonine by utilizing nitrogenous compounds.[23]

Chronic kidney disease (CKD) burdens ~14% of the adult American population and is a major risk factor for developing cardiovascular disease (CVD).[24] Patients with advanced CKD and end stage renal disease (ESRD) on dialysis suffer from high annual mortality rates that reach 25%. Of these deaths, more than half will be the result of cardiovascular causes.[25–27] The increased CVD risk is only partially explained by traditional cardiovascular risk factors, with increasing evidence now pointing towards non-traditional participants such as inflammation, oxidative stress, and endothelial dysfunction.[28–31] A growing body of data has shown the gut microbiomes of CKD patients and experimental animal models are altered.[32,33] In this

review, we will present the current knowledge of changes in the gut microbiota in response to CKD, then elaborate on the proposed mechanisms by which CKD-associated dysbiosis may increase the CVD risk.

Dysbiosis in CKD

There is an expanding research base supporting an altered microbiome in CKD.[32–41] These shifts have been attributed to several factors, including the underlying cause for renal dysfunction (e.g. diabetic kidney disease, glomerulonephritis),[42,43] therapeutic interventions common in CKD patients (e.g. antibiotics and immunotherapy), CKD-specific therapeutic strategies such as iron supplementation and phosphate binders,[44] and dietary restrictions.[18,45–47] It has been long hypothesized that increased intestinal urea concentrations in response to elevated blood urea nitrogen (BUN) together with increased colonic transit time alter the carbohydrate-to-protein balance, producing the noted dysbiosis in CKD.[32,48–50] These hypotheses were supported by the findings of increased urease producing bacteria in patients with CKD and experimental animal studies.[32,41,51–53] Recently, we have also shown an increase in urease producing bacteria in mice with surgically induced CKD.[41] However, we were unable to mimic the CKD-associated changes in the microbiome through oral urea supplementation alone, indicating that intestinal urea concentrations may not be the only factor impacting CKD-associated dysbiosis. This observation needs to be approached carefully. For instance, mice receiving urea supplementation in our experiment were not subjected to 5/6 nephrectomy, limiting the occurrence of other CKD-associated pathophysiological phenomena such as alterations in intestinal pH and increased colonic transit time. Further work is required to better understand microbiome dysregulation in CKD.

The effect of dysbiotic changes in CKD on CVD risk

Figure 1 summarizes the mechanisms by which CKD-associated dysbiosis increases the atherosclerosis and CVD risk.

Gastrointestinal barrier and inflammation

Tight junctions between intestinal epithelial cells play a pivotal role in forming and regulating the gastrointestinal (GI) barrier.[54] Past work by Vaziri et al.[55] has detailed the GI tight junction protein abnormalities in patients with ESRD and experimental animal models of CKD. They demonstrated that CKD-induced experimental rats had diminished transcellular tight-junction proteins. This was present alongside an accumulation of inflammatory cells in the lamina propria and increases in serum monocyte chemoattractant protein-1 (MCP-1) concentrations, which suggested the occurrence of systemic inflammation.[55] They also conducted a large scale histological study showing evidence of chronic inflammation throughout the gut in a population of hemodialysis patients.[56]

Further work assessed the impact of tight junction integrity utilizing the human-derived T84 colorectal carcinoma cell line, which develop into monolayers that exhibit tight junctions.[57,58] Exposure to hemodialysis plasma obtained from ESRD patients resulted in decreased expression of the tight junction proteins zonula occludens-1 (ZO-1), occludin, and claudin-1 in addition to increases in epithelial permeability as evidenced by decreased trans-epithelial/endothelial electrical resistance (TEER) measurements.[59] Subsequent work in the same study compared the impact on the aforementioned tight junction proteins using plasma from either pre- or post-hemodialysis. The results showed a significant inhibitory effect of the post-hemodialysis plasma compared to the pre-, indicating a possible role of elevated urea and other metabolites on the GI epithelium integrity.

To confirm this hypothesis, the group later conducted a study evaluating the direct effect of urea alone and urea combined with urease on TEER measurements and tight junction protein expression.[60] Urease was included because urea hydrolysis in the intestinal lumen by urease-expressing microbes generates ammonia, which is then converted to ammonium hydroxide. This latter metabolic byproduct was proposed to contribute to intestinal epithelial cell toxicity and the subsequent decreases in tight junction proteins. Their results demonstrated a concentration-dependent decline proportional to urea concentration, which was potentiated with the addition of urease. Concentration-dependent decreases were observed in claudin-1, occludin, and ZO-1.[60]

These studies provide evidence that the intestinal uremic milieu observed in CKD patients may result in the loss of tight junction proteins, compromising the integrity of the intestinal barrier.[60] Accumulating data suggest gut leakage of microbial components into the systemic circulation may be a possible cause of systemic inflammation seen in CKD and a contributor to CVD risk.[61] The 5/6 nephrectomy CKD model resulted in an increased expression of claudin-2 and decreased expression of claudin-1.[62] Claudin-2 epithelial expression is increased in different diseases including inflammatory bowel disease, immune mediated diseases, and recently CKD.[62,63] This pattern of tight junction protein expression results in increased intestinal permeability and might partially explain the CKD-related inflammatory state. Claudin-2 plays a role by forming channels and the paracellular transport of sodium, potassium, and fluid in both intestinal epithelial cells and renal tubular cells. In the intestinal epithelia, it is expressed along the crypt-villus axis.[64] The leaky gut/bacterial translocation hypothesis in dialysis patients and experimental animal models was supported by the detection of gut bacterial components in the mesenteric lymph nodes of uremic rats,[65] the peripheral blood of hemodialysis patients (bacterial DNA extraction from blood and dialysate),[66] and endotoxemia in CKD patients.[67–69] This is believed to directly increase CVD risk as intestinal bacterial DNAs were observed in atherosclerotic lesions,[70,71] and their presence is associated with plaque instability.[72,73]

Cross-sectional analysis of patients with ESRD on hemodialysis resulted in the detection of bacterial DNA in blood

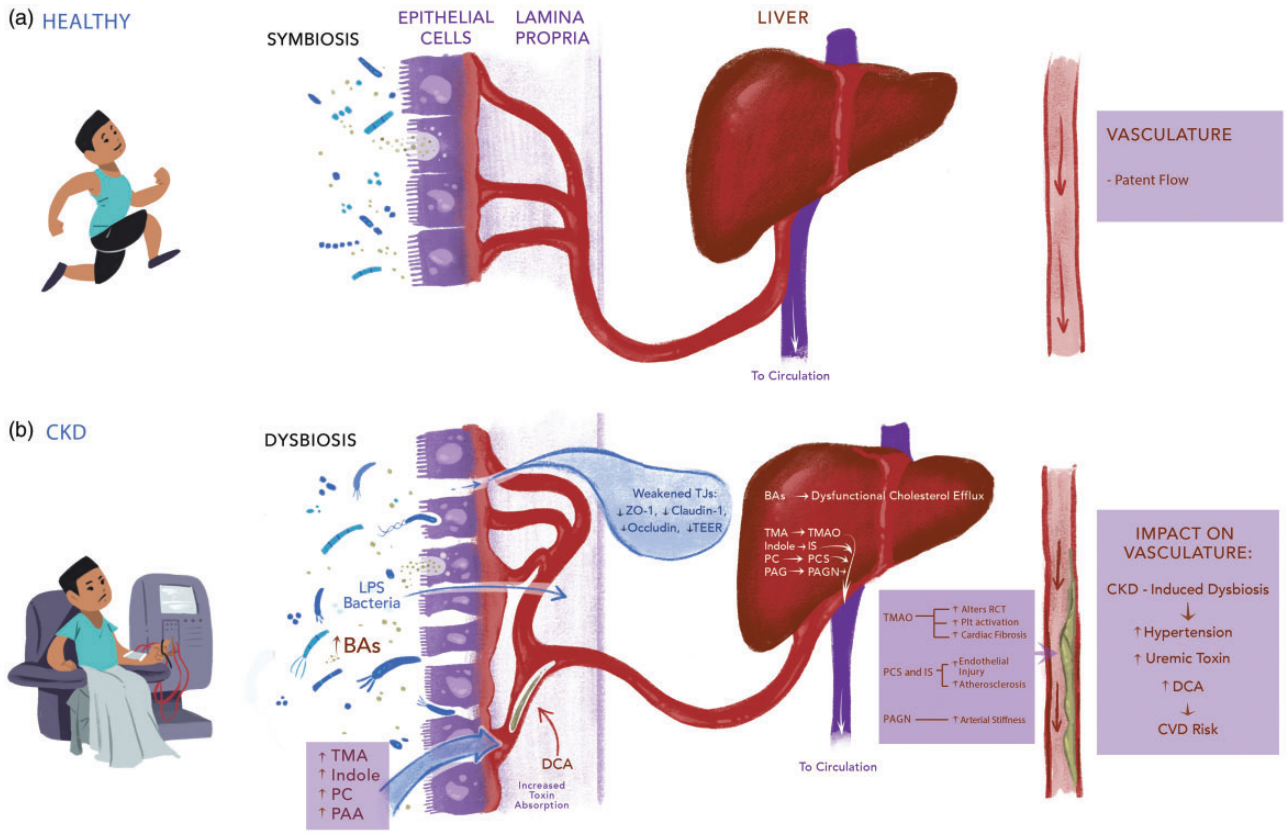

Figure 1. Intestinal microbiome dysbiosis in chronic kidney disease (CKD) promotes cardiovascular disease. (a) Under symbiotic conditions, the intestines represent the location of an ancient, beneficial bond between the local microbiome and the host. (b) Several factors in CKD prompt a detrimental collapse in the host-microbiome relationship, resulting in a dysbiotic state that may instigate the incidence and progression of CVD. First, CKD is associated with localized increases in intestinal permeability, such as those due to the cytotoxicity arising from elevations in luminal urea and its eventual generation of ammonium hydroxide. Impaired tight junctions have been observed in several studies, characterized by decreases in zonula occludens-1 (ZO-1), occludin, and claudin-1 expression in conjunction with decreased transepithelial/endothelial electrical resistance (TEER) measurements. The weakened epithelial barrier permits the passage of bacterial toxins such as lipopolysaccharides (LPS), or even bacteria themselves, which can incur both localized inflammation as well as increased LPS plasma concentrations in CKD. A second influence is that systemic bile acid (BA) concentrations and their intestinal absorption are increased in CKD, leading to negative impacts on hepatic cholesterol handling. Of note, the metabolism of chenodeoxycholic acid, specifically, to deoxycholic acid (DCA) is facilitated by bacterial enzymes. DCA concentrations are nearly doubled in CKD and have been linked to artery calcification. Patients with late-stage CKD also have higher rates of hypertension, which may in part be attributed by shifts in the microbiome. Lastly, elevations in uremic toxins originating from the microbiome may directly increase cardiovascular (CVD) risk, including trimethylamine (TMA), indole, *p*-cresol (PC), and phenylacetic acid (PAA). Once absorbed, these compounds are metabolized within the liver to trimethylamine-*N*-oxide (TMAO), indoxyl sulfate (IS), *p*-cresol sulfate (PCS), and phenylacetylglutamine (PAGN), respectively. They then go on to increase CVD risk by multiple processes like stimulating cardiac fibrosis, endothelial injury, and platelet activation. Plt: platelet; RCT: reverse cholesterol transporter.

samples of 20% of cases.[74] This was associated with increased inflammatory markers (C-reactive protein and IL-6) in ESRD patients with bacterial translocation in comparison to the remaining ESRD patients.[74] *Klebsiella spp., Proteus spp., Escherichia spp., Enterobacter spp.,* and *Pseudomonas spp*. were notably detected in the blood of ESRD patients. Of interest, earlier studies detected bacterial signatures of *Proteus vulgaris* and *Klebsiella pneumoniae* among others in the atherosclerotic lesions in patients with coronary heart disease.[71] Similarly, the relative abundance of *Escherichia coli, Klebsiella spp.,* and *Enterobacter aerogenes* was notably increased in the gut microbiome of patients with coronary heart disease compared to control.[75] These observations (illustrated in Figure 2) of a defined alteration in the CKD microbiome, coupled with the presence of these bacterial DNA signatures in atherosclerotic plaques and the experimental evidence of aberrant intestinal integrity, suggest a pivotal role of dysbiosis, accompanying the leaky gut and inflammation in the development of CVD and plaque instability (*i.e.* acute cardiac events).

Hypertension

Hypertension is not only one of the leading causes of CKD, its prevalence increases drastically with worsening renal function[76]; 40 to about 65% of patients with early stages of CKD suffer from hypertension,[77] and it has been proven extensively that tight blood pressure control improves renal, cardiac, and patient outcomes.[76,78–82] Emerging lines of evidence suggest that the gut microbiome might exert an impact on systemic blood pressure which is highlighted by several recent reviews.[83–85] Gut microbiome studies of pre-hypertensive and hypertensive human populations paralleled spontaneously hypertensive rat models,[86] which displayed decreased microbial richness and diversity with an increased *Firmicutes/Bacteroidetes*

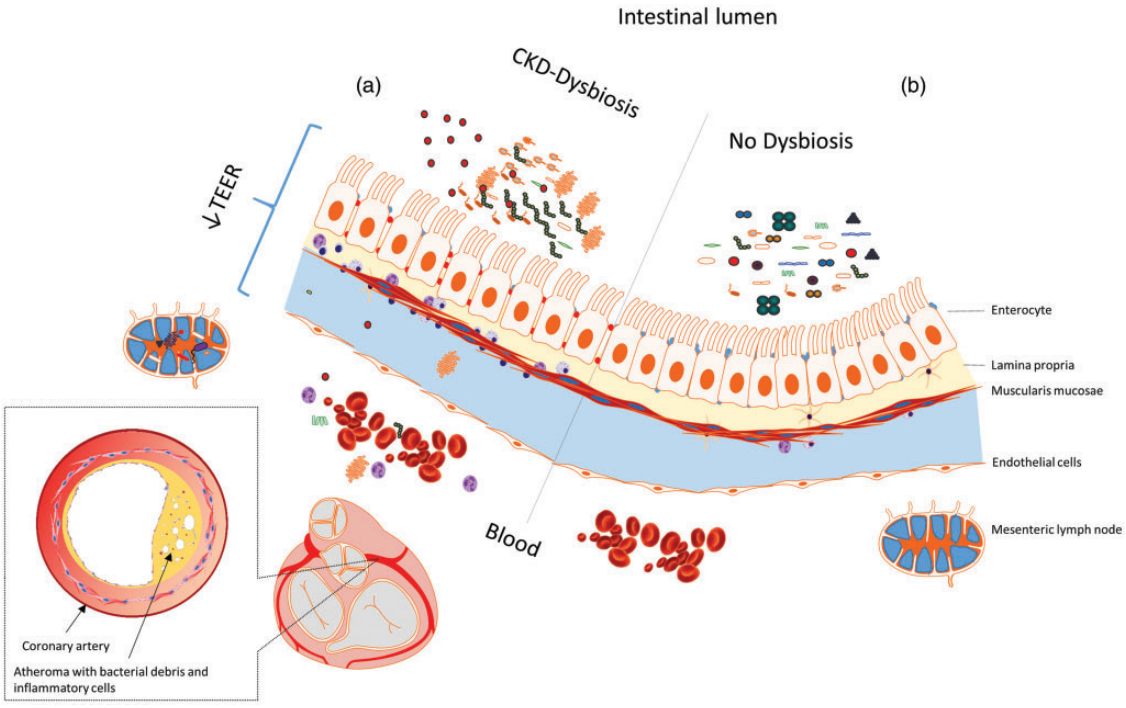

Figure 2. CKD -associated dysbiosis effects on enterocyte integrity and permeability. Panel (a) depicts the pathophysiological changes in response to chronic kidney disease (CKD) in comparison to healthy subjects (b). CKD results in a decreased α-diversity (the variety of the bacterial species) along with changes in β-diversity (expansion/decrease of certain bacteria). The tight junction proteins claudin-1, ZO-1, and occludin (blue dots in the tight junction within the enterocyte paracellular space) are decreased, along with an increase in claudin-2 (red dots in the tight junction at the enterocyte paracellular space) in response to CKD-dysbiosis. This profile leads to the formation of paracellular channels, increased ion and fluid movement from the intestinal lumen (leaky gut), and decreased transepithelial/transendothelial electrical resistance (TEER). Bacterial translocation, or the presence of bacteria in the blood stream and mesenteric lymph nodes, ensues. The result is immune cell activation, invasion of the lamina propria, and a chronic subtle inflammatory state. Chronic inflammation is a pro-atherosclerotic state. This, coupled with the atheroma plaque instability and the existence of bacterial debris in the atherosclerosis lesions, increase the risk of plaque rupture and acute cardiac ischemic events. For simplicity, the schematic does not include changes noted in crypts and villi in response to CKD-dysbiosis.

ratio.[87] Microbiome shifts in response to modulators such as dietary fiber, probiotics, or a high salt diet resulted in changes in systemic blood pressure.[83] The latter connection was further enhanced by the identification that high-salt diet decreased *Lactobacillus spp.* in mouse and man, and whose replacement in hypertensive mice significantly reduced the blood pressure elevations.[88] Likewise, fecal transplantation from hypertensive individuals to germ-free mice was able to elevate blood pressure.[86]

Despite emerging evidence for a link between hypertension and the microbiome, specific mechanisms for how CKD-induced dysbiosis may contribute to hypertension remain sparse. One potential player may be short chain fatty acids (SCFAs), which have the ability to influence blood pressure via G protein-coupled receptors (GPCRs). For example, propionate stimulation of the olfactory receptor 78 (Olfr78) expressed in the renal vasculature results in renin secretion.[89] However, this SCFA attribute remains poorly defined because it is unclear why SCFA-mediated activation of another GPCR, Gpr41, decreases blood pressure.[90] Additional work in this area is warranted to better characterize the relationship between circulating SCFAs and systemic blood pressure in CKD. This is portrayed by the actualities that these GPCRs are found throughout the body and have varying SCFA concentration-effect profiles.[91,92]

A second potential connection between dysbiosis in CKD and increased CVD risk is the concept that inflammation plays a combinatorial role in hypertension and CKD progression. Past work has demonstrated relations between low-grade inflammation and CKD progression[93,94] together with immune-dependence for angiotensin II-directed hypertension.[95–97] Studies employing angiotensin II-infused germ-free mice showed that the gut microbiota may play a role in the potentiation of angiotensin II-associated hypertensive effects and vascular dysfunction.[95] This effect was attributed to the mitigation of immune cell infiltration and cytokine production in germ-free mice, suggesting microbiome-driven immune conditioning may play a role in angiotensin II promoted hypertension. Consistent findings were noted in a systematic review and meta-analysis, where systolic and diastolic blood pressure both decreased after probiotics usage in several clinical trials.[98] Elevated blood pressure may also negatively impact intestinal epithelia, whereby intestinal integrity, local inflammation, and microbiome dysbiosis were all noted in spontaneous hypertensive rats. These effects were corrected with antihypertensive captopril therapy, signifying hypertension may contribute to microbiome alterations.[99] Future work connecting dysbiotic shifts in CKD and angiotensin II-mediated hypertension represent an intriguing avenue, as does the notion that the

relationship between microbial dysbiosis and hypertension may go both ways.

Bile acid metabolism

Synthesized from cholesterol by hepatocytes, the two primary bile acids (BAs) chenodeoxycholic acid and cholic acid undergo conjugation in the liver that leads to their nearly complete ionization at physiological pH. The resultant bile salts then get stored in the gallbladder where they are subsequently secreted into the duodenum to aid in the absorption of dietary lipids. Active and passive transport processes predominantly in the ileum facilitate the efficient intestinal reabsorption (~95%) of BAs back into the blood that eventually sees their return to bile via the liver, completing the cycle of enterohepatic recirculation.[100–104] Over the course of their route, BAs encounter the intestinal microbiome which conducts several metabolic transformations that include deconjugation and dehydroxylation. The extent of bacterial metabolism is not trivial, which is exemplified in comparisons of fecal and biliary BA compositions.[101]

Total circulating BAs are elevated in both CKD animal models and patients.[104–106] Increased attention is being directed towards one specifically: deoxycholic acid (DCA). This secondary bile acid, or bacterially-modified BA, is generated via bacterial dehydroxylation of cholic acid via the 7α-dehydroxylase enzyme.[107] Elevated concentrations of circulating DCA were recently associated with coronary artery calcification in individuals with moderate CKD.[108] Therefore, CKD-induced dysbiosis may result in an elevated DCA exposure, which could directly lead to higher CVD risk. However, a notable ambiguity between these associations is that bariatric surgery can also increase both total BA and DCA serum concentrations.[109,110] Seeing as the efficacy of this procedure has been partly attributed to increased circulating BA concentrations,[109] and bariatric surgery is directly linked to better health outcomes in diabetes,[109] CVD risk,[111] and even CKD risk.[112] A better understanding of the molecular mechanisms underpinning heightened BAs/DCA in CKD and their potential to increase CVD risk are required.

Bile salts not only emulsify fat. They have been demonstrated to act as efficacious signaling molecules that can stimulate self-inhibitory feedback loops to decrease BA synthesis as well as serve as pivotal effectors in systemic metabolism.[100,101,109,113] BAs are endogenous ligands of the nuclear Farnesoid X receptor (FXR), a BA-activated transcription factor mostly found in the liver, kidneys, and intestines.[114] Hepatic and renal FXR expression have been shown to progressively decrease with worsening diabetes mellitus and diabetic kidney disease.[115–117] FXR agonism, either alone or together with a second BA effector (Takeda G protein coupled receptor, TGR5),[118] has been targeted for a plethora of treatments that include CKD and atherosclerosis.[109,115,117–123]

Several reports exist detailing a microbiome-specific impact on FXR activation via secondary BAs (reviewed in Wahlstrom *et al.*).[100,124–126] For example, Sun *et al.*[124] recently reported that metformin treatment increased concentrations of the novel FXR antagonist glycoursodeoxycholic acid by decreasing the abundance of *Bacteroides fragilis*, which is responsible for its hydrolysis. What is currently unknown is if a similar scenario occurs in CKD whereby dysbiosis influences BA metabolism and the expression of FXR. Relating to CVD risk, *Fxr* knockout mice fed regular chow exhibited an increase in serum BA and increased cholesterol 7α-hydroxylase (*Cyp7a*) amounts, a key enzyme in bile acid synthesis whose expression is inhibited by FXR.[127,128] This may be important in the development of CVD as both FXR and CYP7A1 are also associated with cholesterol efflux, where their dysregulation (decreased FXR, increased CYP7A1) could be contributing to CVD risk via elevated concentrations of systemic cholesterol and triglycerides.[121,127,129]

FXR also appears to play an important protective role in the negative regulation of vascular calcification, as evident by studies in apolipoprotein E (*Apo*E) knockout mice subjected to 5/6 nephrectomy. FXR agonists inhibited the phosphate-induced mineralization and triglyceride accumulation in both *Apo*E knockout CKD mice aortas, and bovine calcifying vascular cells.[130] What remains to be clarified is if FXR expression is decreased in all CKDs, as a study in rats with experimentally induced chronic renal failure demonstrated no change in hepatic, intestinal, or renal *fxr* mRNA expression.[104] Additionally, tissue-specific effects of FXR activation in CKD would need to be addressed because intestinal FXR increases metabolic syndrome susceptibility, highlighting the poor understanding of FXR signaling across multiple organs in pathophysiology.[109,131]

Advanced glycation end products

The amounts of advanced glycation end products (AGEs) are markedly elevated and directly associated with worse outcomes in patients with CKD,[132,133] diabetes mellitus,[132] and CVDs.[134] There is a strong body of evidence linking increased AGE concentrations with cardiovascular events through multiple mechanisms including cross-linking properties and increased vascular stiffness, stimulation of pro-inflammatory pathways such as Nuclear factor κ-light chain-enhancer of activated B cells (NF-κB), the glycation of the atheroma collagen which accelerates atherosclerotic lesion progression, and increased oxidization susceptibility.[134,135] Seventy percent of dietary AGEs are not absorbed and arrive at the colon, where they may react with the bacterial microbiota[136,137] and result in alteration in the composition of the gut microbiota.[39,138,139]

In recent years, studies have emerged linking AGE-rich diets with changes in gut microbiota and establishing a possible role by which AGEs may contribute to worse CVD outcomes.[140–142] Mice fed a high AGE diet for eight months exhibited a unique gut microbiome defined by a decrease in α-diversity,[142,143] and changes in β-diversity on all levels (phyla, family and genera levels).[143] The high dietary AGE group revealed an increase in *Cyanobacteria* phylum. On the family level, *Porphyromonadaceae*, *Prevotellaceae*, *Helicobacteraceae*, and *Alcaligenaceae* were increased, while *Bacteroidaceae*, *Lachnospiraceae*,

Desulfovibrionaceae, *Rikenellaceae*, *Anaeroplasmataceae* were decreased in relative abundances. Eighteen genera were significantly different in response to intervention.[143] Importantly, *Bacteroides Spp*. was decreased, which is a genus that has been shown to exert cardiovascular protective properties.[144,145] It ferments carbohydrates resulting in the production of an array of volatile fatty acids that are used by host intestinal cells and other gut bacteria as a source of energy, which results in favorable energy and metabolism profiles.[18,146] Interestingly, high AGE fed mice exhibited an increase in the uremic toxin p-cresol (relevance discussed below).[143] It is important to note that high dietary AGE mice were not subjected to any renal injury. These findings strongly support the notion that dietary AGE-associated gut microbiota dysbiosis results in increased production of uremic toxins.

Changes in microbiota secondary to increased AGEs in patients with renal disease may increase CVD risk through several possible mechanisms. High AGE-related dysbiosis affects the SCFA profile. While studies agreed that dietary AGEs decrease acetate and increase isovalerate and isobutyrate productions, conflicting results were noted in regards to butyrate.[142,143] Colon structure and integrity were distorted in response to AGEs. This was evident by the loss of intestinal crypts, goblet cell depletion, and increased dysplasia and thickening of colonic wall epithelia.[142] At the same time, increased colonic permeability resulted from AGE exposure, which was indicated by the loose cellular arrangement histologically along with a decreased expression of occludin and ZO-1. However, to our knowledge, no study has yet to mechanistically link AGE-associated dysbiosis with increased inflammatory markers. It is highly probable that AGE-related increases in inflammation can partially be attributed to its effects on microbiota and gut permeability, leading to increased CVD risk.[147]

SCFAs

SCFAs are mainly produced by the intestinal flora from carbohydrate fermentation, with acetate (~50% of all SCFAs), propionate, and butyrate collectively accounting for more than 90% of all SCFAs.[148] Patients with advanced CKD and those with ESRD are advocated to follow a strict dietary restriction that generally decreases potassium consumption. Limiting the consumption of potassium-rich food, such as fruits, vegetables, and high fiber containing food can decrease the colonic carbohydrate/protein availability, resulting in dysbiosis that disfavors SCFA-producing bacteria.[32,45,48–50] As expected, patients with CKD were demonstrated to have alterations in the amount of SCFA-producing microbiota.[45]

The SCFAs acetate, butyrate, and propionate have been reported to have anti-inflammatory and histone deacetylase properties.[149] Therapy with SCFAs improved renal dysfunction in an ischemic acute kidney injury model, and lowered the levels of local and systemic inflammation, oxidative stress, and cell apoptosis.[150] Butyrate and other SCFAs serve as a primary energy source for epithelial cells of the intestinal tract in a preferential manner.[151,152] They also dampen the pro-inflammatory response in intestinal epithelial cells.[153]

This indicates that alterations in SCFAs secondary to CKD associated dysbiosis might negatively impact the integrity of intestinal epithelial cells, further worsening the pro-inflammatory state. Restoration of SCFAs using a high-fiber diet in an experimental autoimmune hepatitis animal model resulted in improved intestinal histological structures measured by crypt number and depth, ameliorated intestinal track permeability, and reduced bacterial translocation.[154] Similarly, direct administration of butyrate resulted in decreased intestinal permeability, improved intestinal histology (villus depth), and decreased inflammation after administration of 5-fluorouracil.[155] The beneficiary effects of SCFAs in CKD are not only limited to their favorable effects on the intestinal cells, they also provide a large spectrum of metabolic, anti-diabetic, anti-inflammatory, and anti-hypertensive effects resulting in overall favorable renal and cardiac outcomes.[149–153]

Microbiota-derived uremic toxins and CVD risk

p-Cresol sulfate and indoxyl sulfate

CKD-related dysbiosis is associated with amplified indoxyl sulfate (IS) and p-Cresol sulfate (PCS) production.[156] IS is generated from dietary tryptophan metabolism by bacterial tryptophanases into indole, which is absorbed and converted to IS by the host liver. PCS is produced as a byproduct of tyrosine and phenylalanine colonic fermentation into p-cresol, which later is converted to PCS by the host hepatic and intestinal epithelial cells' aryl transferase.[157] IS and PCS are highly protein bound and their elimination greatly depends on various renal tubular transporters.[158–160] Both circulate systemically via the non-covalent binding to albumin at the Sudlow binding site II.[158,161] In those with intact renal function, the organic anion transporters (OAT) 1, 3, and 4 play a pivotal role in the clearance of these uremic toxins. Due to the combination of increased uremic toxin production via CKD-related dysbiosis and the gradual worsening of renal function, IS and PCS will accumulate secondary to the loss of renal mass and the inability of the failing kidneys to actively eliminate these toxic metabolites.[161,162] This effect is compounded in patients with ESRD, where uremic toxin removal by hemodialysis is marginal because of the high fraction that is bound to albumin.

IS and PCS have both been linked to adverse renal, cardiovascular, and mortality outcomes in CKD patients[163–165] and have been directly associated with vascular endothelial injury[166] and atherosclerosis severity.[167] Researchers have looked into the mechanisms by which these uremic toxins exert unfavorable clinical outcomes. Though an in-depth description of these pathways is beyond the scope of the current review, it is important to note that PCS and IS have been implicated in the worsened CVD risk in CKD through different mechanisms that include: oxidative stress and endothelial dysfunction in CKD patients,[168] stimulation of the transforming growth factor-β (TGF-β) pathway, tubulointerstitial fibrosis,[169] and insulin resistance and

dyslipidemia.[170] *In vitro* experiments confirmed pro-inflammatory changes in cultured proximal tubular cells in response to PCS and IS co-culture. Interestingly, cytokine networks and the alteration of intracellular inflammatory signaling pathways were similar in regards to IS and PCS, indicating a possible synergistic effect when both are elevated.[171]

The rich *in vitro* and experimental animal model evidence in support of poorer outcomes in response to IS and PCS exposure have not yet been efficiently translated to strong and conclusive findings in clinical studies. Although observational and retrospective clinical studies indicated increased CKD progression, mortality, and CVD risk in those with elevated IS and PCS levels,[163–165] recent analysis taking into account all confounding factors failed to definitively reach a similar conclusion.[172,173] For example, Lin *et al.*[174] conducted a systematic review and meta-analysis in order to evaluate the association between these uremic toxins and outcomes. They noted an increased mortality risk in CKD patients with elevated PCS and IS levels. However, only PCS was associated with CVD risk in this population. Based on the experimental evidence and this report, it is logical to assume that interventions aimed at decreasing the amount of circulating PCS and IS would confer favorable outcomes in CKD patients. Early and small studies utilizing the carbon adsorbent AST-120 confirmed the therapeutic potential of decreasing uremic toxin amounts, along with a possible effect on delaying the initiation of renal replacement therapy if given to pre-dialysis CKD patients.[175]

However, a better designed and larger trial that was randomized and placebo controlled could not confirm the benefit of AST-120 on disease progression.[176] It remains to be seen if these negative findings were attributed to the slow disease progression over the study duration, slow advancement of the renal disease (mean serum creatinine concentrations were ~3 mg/dL), or the fact that AST-120 is a general resin and might have resulted in an unintentional chelation of vitamins and other essential elements, or even possibly that elevation levels of uremic toxins are simply indicative of disease severity. This notion is supported by the fact that although AST-120 might affect CKD progression, it does not affect mortality or CVD risk in pre-dialysis CKD patients.[175]

Trimethylamine-*N*-oxide

Trimethylamine-*N*-oxide (TMAO) is an end product of the bacterial metabolism of choline/phosphatidylcholine and L-carnitine with links to atherosclerosis and higher mortality in CKD and non-CKD populations, plus experimental animal models of atherosclerosis.[177–179] Abundant data suggest that dysbiosis is directly responsible for higher TMAO levels observed in CKD.[38] TMAO appears to be involved in continuous renal fibrosis that is observed in CKD.[180] It increases CVD risk in CKD patients through different mechanisms. TMAO upregulates cholesterol scavenger receptors on macrophages,[177] encourages platelet activation by boosting intracellular Ca^{2+} release,[181] and leads to maladaptive ventricular remodeling and cardiac fibrosis.[182] On the cellular level, TMAO increases fibrosis through TGF-β/p-Smad3 pathway activation, and stimulation of the renin-angiotensin aldosterone pathway.[169]

Phenylacetylglutamine

Phenylacetylglutamine (PAGN) is produced as a result of phenylalanine fermentation to phenylacetic acid (PAA) by gut microbes and the subsequent conjugation with glutamine by both hepatocytes and the kidneys. PAGN is then excreted in the urine.[183] This process removes glutamine from the urea cycle and participates in nitrogen scavenging activities. Elevated concentrations of circulating PAGN were associated with advanced CKD [184] and studies suggest an association with elevated CVD risk in non-dialysis CKD patients.[185,186] Furthermore, PAGN levels have been recently linked to increased overall mortality and CVD in patients with different stages of impaired renal function.[184] The direct and linear correlation between PAGN concentrations and increased pulse wave velocity increased Framingham cardiovascular risk scores, and arterial ageing and stiffness are proposed as possible mechanisms by which PAGN increases CVD.[187,188] Although the kidneys efficiently clear PAGN and accumulation may be due to declining renal function, urinary excretion studies in non-dialysis CKD patients have shown that the gut microbiome may contribute to increased production or absorption.[184,189]

However, the exact mechanism by which PAGN increases CVD risk is still not fully understood as the currently available evidence is limited to epidemiological trials. It is quite plausible that changes in serum PAGN concentrations seen in these trials reflect advanced CKD-associated dysbiosis and its overall deleterious impact on inflammation and intestinal epithelial integrity. Additionally, there could be increased production of unmeasured bacterial-derived metabolites other than PAGN. Or, these observations may simply follow the worsening renal disease and the decline in residual reserved renal function. This is evident by the lack of association between cardiovascular outcomes and PAGN levels in patients on hemodialysis.[172]

Conclusion

CVD prevalence and disease burden have been increasing worldwide.[190] It is the number one cause of death globally, necessitating the launch of the Global Hearts Initiative in 2016 as a joint effort between the World Health Organization and the United States Centers for Disease Control and Prevention. CKD is a prevalent risk factor for CVD development.[24–27] Here, we have outlined the evolving evidence on how CKD-associated changes in the gut microbiome may increase CVD risk in CKD patients. Generation of the uremic toxins IS and PCS in addition to alterations in intestinal permeability represents topics in the field that have been studied more thoroughly. More recent concepts garnering new focus include microbiome-dependent regulation of systemic hypertension, modifications of BA metabolism, and AGE-related dysbiosis. Because of the diversity in abnormalities occurring in

CKD, it is likely that several microbiome-associated factors combine together to elevate CVD risk in the CKD patient population. It is this attribute that exemplifies the importance of future research into these alternative pathways.

It should be noted that this assortment in pathology also leads to experimental complications because it is often difficult to evaluate multiple pathophysiologic processes in one study. Future work should be directed towards identifying the contributions of multiple microbiome-associated processes on CKD progression and CVD risk, such as the impact of a leakier gut on the absorption of bacterially derived uremic toxins or BAs. Additionally, several key unknowns remain for the majority of topics discussed. The time and effect dependence is still relatively uncharacterized between microbiome dysbiosis and CKD progression, let alone CVD onset. It will be imperative to measure microbiome changes and any ensuing pathologic processes at various stages of CKD. For example, when exactly does intestinal permeability increase in CKD, and how does this relate to dysbiosis and microbiome-specific processes on CKD advancement/CVD risk at each stage of the disease? Furthermore, it remains to be seen clinically if therapeutic strategies specifically manipulating the microbiome can infer efficacy towards halting CKD progression. It will also be vital to ensure that any delivered bacteria intended for therapy is/are capable of surviving and establishing themselves in the modified intestine of a CKD individual.

In conclusion, dysbiotic fluctuations in the human microbiome continue to materialize as a predominant factor in human health. Future work will thus provide important insight into the impact of microbial modifications in the development and progression of CKD, and its concomitant CVD risk.

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DECLARATION OF CONFLICTING INTERESTS

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