Microbiome and Cardiovascular Disease in CKD

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Abstract

Patients with CKD exhibit a disproportionate burden of cardiovascular mortality, which likely stems from the presence of unique, nontraditional risk factors that accompany deteriorating kidney function. Mounting evidence suggests that alterations to the intestinal microbiome in CKD may serve as one such risk factor. The human intestinal tract is home to >100 trillion micro-organisms made up of a collection of commensal, symbiotic, and pathogenic species. These species along with their local environment constitute the intestinal microbiome. Patients with CKD show intestinal dysbiosis, an alteration of the gut micro-organism composition and function. Recent evidence links byproducts of intestinal dysbiosis to vascular calcification, atherosclerosis formation, and adverse cardiovascular outcomes in CKD. CKD-associated intestinal dysbiosis may also be accompanied by defects in intestinal barrier function, which could further enhance the negative effects of pathogenic intestinal bacteria in the human host. Thus, intestinal dysbiosis, defective intestinal barrier function, and a reduced capacity for clearance by the kidney of absorbed bacterial byproducts may all potentiate the development of cardiovascular disease in CKD. This narrative review focuses on microbiome-mediated mechanisms associated with CKD that may promote atherosclerosis formation and cardiovascular disease. It includes (*1*) new data supporting the hypothesis that intestinal barrier dysfunction leads to bacterial translocation and endotoxemia that potentiate systemic inflammation, (*2*) information on the accumulation of dietary-derived bacterial byproducts that stimulate pathways promoting atheromatous changes in arteries and cardiovascular disease, and (*3*) potential interventions. Despite great scientific interest in and a rapidly growing body of literature on the relationship between the microbiome and cardiovascular disease in CKD, many important questions remain unanswered.

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Introduction

CKD affects 14% of adults in the United States (1). Patients with CKD are at a far greater risk of developing cardiovascular disease compared with the general population (2), and it is evident that this cardiovascular disease burden is not entirely driven by a higher prevalence of traditional risk factors. Accumulating evidence supports metabolites produced by intestinal microbes as nontraditional risk factors for cardiovascular disease in CKD. Thus, scientific interest is rapidly evolving to better understand the contribution of the intestinal microbiome to cardiovascular disease in patients with CKD. This review focuses on new evidence linking gut-derived microbial byproducts to atherosclerosis and cardiovascular outcomes in CKD.

Microbiome Alterations in CKD

The human intestinal tract is home to >100 trillion micro-organisms, which consist of a collection of commensal, symbiotic, and pathogenic species contributing to a local ecologic community termed the microbiome. The microbiome aids in food digestion, vitamin biosynthesis, bile acid biotransformation, innate immunity, and intestinal barrier maintenance. In humans, most intestinal bacteria belong to five phyla; however, substantial species-level variability exists and is determined by genetic and environmental factors (3). Recent studies show dysbiosis, which is characterized by shifts in the relative abundance of major bacterial populations, in fecal samples from patients with CKD (4) and patients with ESKD versus healthy controls (5–7). The factors contributing to dysbiosis in kidney disease remain largely undefined; however, dietary restriction (3), use of phosphate binders (8) and antibiotics (3,9), and even CKD (10) itself may play a role.

Intestinal dysbiosis that is observed in CKD and ESKD may increase the production of bacterial byproducts that are rapidly absorbed from the intestinal lumen. When increased absorption is coupled with decreased clearance by the kidneys, levels of gut-derived toxins rise in circulation and may promote cardiovascular disease. Additionally, animal studies suggest the presence of defects in intestinal barrier function in CKD (3). The endotoxemia and systemic inflammation, which could result from intestinal barrier defects, are also established risk factors for cardiovascular disease (11). A comprehensive review of the intestinal microbiome and CKD was recently published by Ramezani and Raj (3). In this review, we focus on new evidence that supports the mechanisms by which intestinal dysbiosis associated with kidney disease potentially contributes to cardiovascular disease (Figure 1, Table 1) and discuss potential interventions (Table 2).

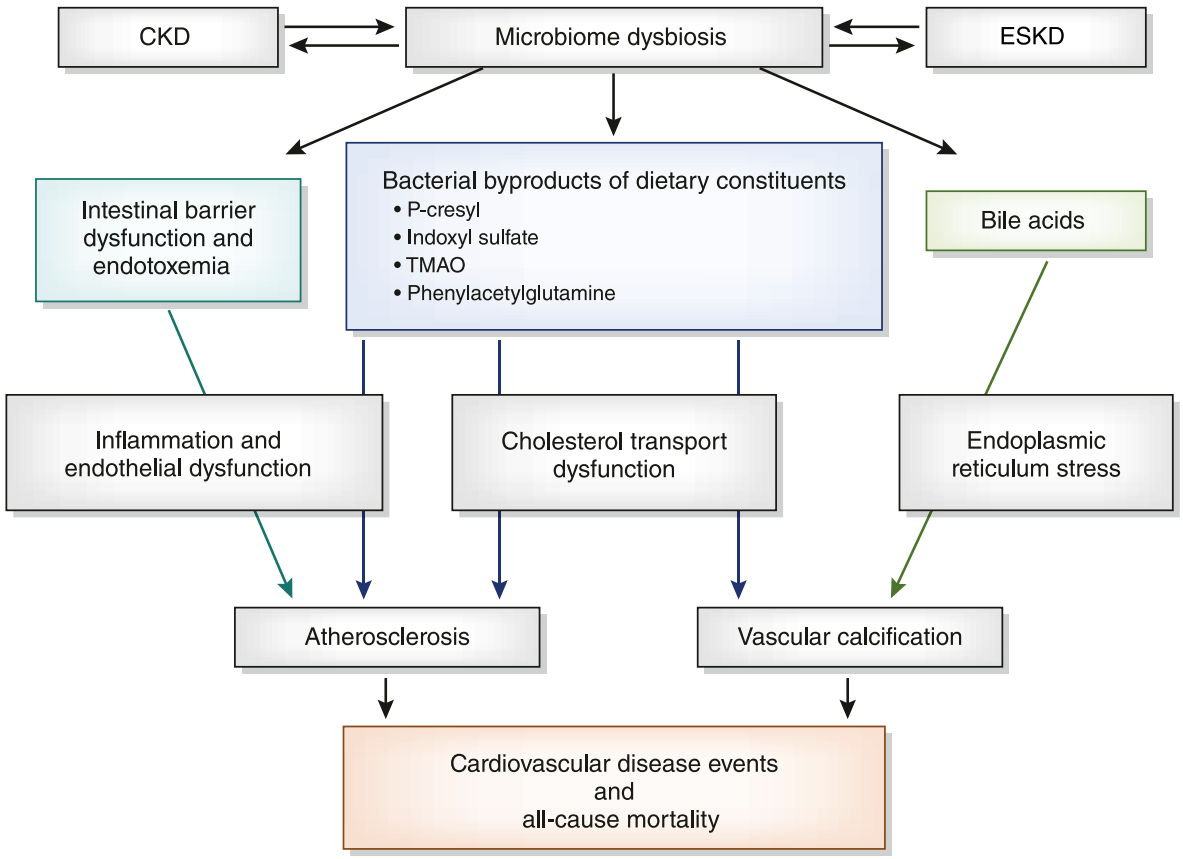

Figure 1. | **Mechanisms by which microbiome dysbiosis in kidney disease may lead to cardiovascular disease.** TMAO, trimethylamine-*N*-oxide.

Dysbiosis, Intestinal Barrier Disruption, Endotoxemia, and Inflammation

Systemic inflammation is a consequence of reduced kidney function and a nontraditional risk factor for cardiovascular disease (11). Although the mechanisms triggering innate immunity and associated inflammation in CKD are debated, one hypothesis is that low levels of bacterial endotoxin are introduced into the bloodstream of patients with CKD and stimulate these pathways. Endotoxemia is observed to be more prevalent in patients with CKD and patients with ESKD versus controls (3), and it is associated with cardiovascular disease in CKD (3) and non-CKD populations (11). In addition to being proinflammatory stimuli, endotoxins directly cause endothelial dysfunction, one of the earliest findings in atherosclerosis (11).

The mechanisms whereby bacterial endotoxin is introduced into the circulation in patients with CKD remain unclear. Among patients undergoing dialysis, endotoxemia may result from the dialysis procedure. However, endotoxemia is noted in earlier stages of CKD (3), suggesting the contribution of factors unrelated to dialysis. As reviewed previously, disruption of the normal intestinal barrier may facilitate passage of bacterial endotoxins into the circulation. Translational studies support this hypothesis by showing significant reductions in intestinal epithelial cell tight junction proteins (claudin-1, occludin, and zona occludins 1) in rat models of CKD (3). Likewise, incubating human enterocytes in uremic plasma increases epithelial permeability and reduces tight junction protein expression (3).

New data show a relationship between CKD-related dysbiosis and systemic inflammation in both animals and humans (7,10). Furthermore, animal data suggest that dysbiosis itself alters intestinal barrier function, leading to bacterial translocation and systemic inflammation. More specifically, in a CKD mouse model showing systemic inflammation, endotoxemia, and intestinal dysbiosis, elimination of facultative anaerobic microbiota prevented bacterial translocation and reduced endotoxemia and systemic markers of inflammation (10). However, although dysbiosis in a small ESKD cohort was associated with systemic inflammation, there was no evidence of intestinal inflammation or bacterial translocation (7). If intestinal barrier function is compromised and leads to systemic inflammation, the origin of these changes in humans with CKD remains uncertain.

Bacterial Byproducts of Dietary Constituents

Indoxyl Sulfate and p-Cresyl

Indoxyl sulfate and p-cresyl are two well studied uremic toxins that are byproducts of bacterial metabolism of dietary constituents. Indoxyl sulfate and p-cresyl are excreted by the kidney, and serum concentrations progressively increase as GFR declines. These metabolites are highly protein bound; thus, they are poorly removed by dialysis. Intestinal bacteria tryptophanase enzymes

Table 1. Microbiome-related mediators of cardiovascular disease and poor cardiovascular outcomes in CKD

Cardiovascular Risk Factor and Potential Mediators	Mechanism of Cardiovascular Disease Promotion	Relationship to Cardiovascular Outcomes in Kidney Disease
Intestinal barrier disruption, endotoxemia, and inflammation		
Bacterial endotoxins		
Bloodstream entry facilitated by intestinal barrier dysfunction (3,10) causing systemic inflammation (10)	Innate immunity activation causing inflammation (11)	Higher endotoxin levels among patients with CKD and patients with ESKD compared with healthy controls (3)
Intestinal dysbiosis favoring pathologic bacterial populations (5)	Vascular endothelial dysfunction (3,11)	Endotoxin levels are higher among patients with ESKD and cardiovascular disease compared with those without cardiovascular disease (3) Endotoxin levels and inflammation positively correlate with carotid intimal medial thickness in patients with ESKD (3) Higher endotoxin levels are significantly associated with all-cause mortality in patients with ESKD (3)
Bacterial byproducts of dietary constituents		
Indoxyl sulfate		
Bacterial byproduct of tryptophan metabolism (4)	Induces inflammation and oxidative stress causing vascular endothelial cell injury by (13) leukocyte activation and adhesion, increasing proinflammatory cytokines and reactive oxygen species, and reducing NO production	Positively correlates with measures of vascular dysfunction (14,15) and aortic calcification (15)
	Decreases macrophage cholesterol efflux	Independently associated with cardiovascular disease (15–17) and atherosclerosis (18)
p-Cresyl		
Derived from bacterial fermentation of tyrosine and pheylalanine (4)	Induces inflammation and oxidative stress causing vascular endothelial cell injury by (13) leukocyte activation and adhesion, increasing proinflammatory cytokines and reaction oxygen species, and reducing NO production	Positively correlates with measures of vascular dysfunction (14,15) and aortic calcification (15)
		Independently associated with cardiovascular disease (15–17)and atherosclerosis (18)
TMAO		
Bacterial conversion of L-carnitine and choline to TMA precursor (19–21)	Increases macrophage cholesterol scavenger receptors (19)	Independently associated with all-cause mortality (23,26,27,29)
Shift in dominant intestinal bacteria composition in CKD leads to altered gene expression favoring TMA production (4)	Reduces reverse cholesterol transport (20)	Independently associated with cardiovascular events and mortality (27)
TMA conversion to TMAO by hepatic Flavin mono-oxygenase (22)		Independently associated with greater atherosclerotic burden (23)
		Independently associated with cardiovascular ischemic events (25)
Phenylacetylglutamine	Mechanism not determined	Independently associated with cardiovascular disease events in CKD (31) and ESKD (32)
Bacterial conversion of phenylalanine to phenylacetic acid, which is then conjugated with glutamine		
Bile acids		
DCA	Induces vascular calcification through endoplasmic reticulum stress mechanism (37)	Independently associated with greater coronary artery calcification (39)
Intestinal bacteria biotransformation of primary bile acid (cholic acid) to secondary bile acid DCA (33)		

NO, nitric oxide; TMAO, trimethylamine-*N*-oxide; TMA, trimethylamine; DCA, deoxycholic acid.

Table 2. Therapies that modify the human intestinal microbiome and bacterial byproduct metabolism in kidney disease

Study Design	Intervention	Subjects	Primary Outcome	Secondary Outcomes	Ref.
Adsorbent					
RCT, 1:1 active versus placebo, 189-wk EPPIC1, 170-wk EPPIC2	AST-120	CKD 4, $n=2035$	No change in time to kidney disease progression[a]	No change in time to kidney disease progression,[a] including death	40
Synbiotic					
RCT, 2:1 active versus placebo, 30 d	Probinul Neutra	After kidney transplant, $n=36$	↓ p-Cresol	No change in kidney function, glycemia, lipids, albumin	41
RCT, active versus placebo, 6-wk crossover, 4-wk washout	Synbiotic	CKD 3–4, $n=37$	No change in indoxyl sulfate	↓ p-Cresol; significant change in microbiome; ↑ albuminuria; no change in eGFR, urinary kidney injury molecule-1, serum inflammatory, or oxidative stress biomarkers	42
RCT, 1:1 active versus placebo, 30 d	Probinul Neutra	CKD 3–4, $n=30$	↓ p-Cresol	No change in gastrointestinal symptoms	43
RCT, 1:1 active versus placebo, 2 mo	Synbiotic	ESKD, $n=18$	↑ Stool bifidobacterial counts	↓ Gastrointestinal symptoms	44
RCT, 1:1 active versus placebo, 2 mo	Synbiotic	ESKD, $n=44$	↓ Gastrointestinal symptoms	↓ Markers of malnutrition and serum CRP and TNF-α levels (NS)	45
Prebiotic					
RCT, active versus placebo, 6-wk crossover, 4-wk washout	Arabinoxylan oligosaccharides	CKD 3–4, $n=39$	↓ TMAO; no change in urea, p-cresyl sulfate, p-cresyl glucuronide, indoxyl sulfate, phenylacetylglutamine	No change in urinary excretion of urea, p-cresyl sulfate, p-cresyl glucuronide, indoxyl sulfate, phenylacetylglutamine, TMAO; no change in insulin resistance	46
Sevelamer					
RCT, 1:1 active versus placebo, 3 mo	Sevelamer	CKD 3–5 (predialysis), $n=69$	↓ p-Cresol	↓ LDL and serum phosphorus	8
Dietary fiber					
Single blinding, 12 wk	Dietary fiber	CKD 3, $n=13$	↓ p-Cresol	↑ Stool frequency; no change in quality of life	47
RCT, 1:1 active versus placebo, 6 wk	Dietary fiber	ESKD, $n=40$	↓ Indoxyl sulfate; ↓ p-cresol (NS)	No change in predialysis weight, BUN, serum albumin, prealbumin, CRP, phosphorus, or KDQOL-36	48
Antibiotics					
Observational, 28 d	Single 250-mg oral dose of vancomycin	ESKD, $n=10$	↓ Indoxyl sulfate; ↓ p-cresyl sulfate	Decrease in gut microbiome diversity	9

RCT, randomized, controlled trial; EPPIC1, Evaluating Prevention of Progression of CKD 1; EPPIC2, Evaluating Prevention of Progression of CKD 2; CRP, C-reactive protein; NS, nonsignificant; TMAO, trimethylamine-*N*-oxide; KDQOL-36, Kidney Disease Quality of Life 36 instrument.
[a]Composite of dialysis initiation, kidney transplant, and serum creatinine doubling.

convert dietary tryptophan to indole, which is absorbed and metabolized to indoxyl sulfate in the liver. p-Cresyl is derived from p-cresol, a product of bacterial fermentation of tyrosine and phenylalanine in the colon (3). Although elevated serum concentrations of indoxyl sulfate and p-cresyl in CKD may result from decreased excretion by the kidney, new data suggest that changes in the microbiome favor increased production of these compounds. A potential mechanism for increased formation of these uremic toxins in kidney disease could be epigenetic changes induced by gut dysbiosis, which could alter amino acid metabolism to favor indoxyl sulfate and p-cresyl generation. In support of this theory, new data from a CKD rat model with disordered amino acid metabolism and elevated indoxyl sulfate and p-cresyl showed that shifts in the abundance of specific intestinal bacteria were associated with activation of genes related to amino acid metabolism (12). Another recent investigation observed a greater relative abundance of intestinal bacteria containing enzymes that facilitate the generation of indoxyl sulfate and p-cresyl among patients with ESKD (5).

Both indoxyl sulfate and p-cresyl are purported vascular toxins. Cell culture and animal studies show that these metabolites cause vascular endothelial cell injury by increasing leukocyte activation and adhesion and contributing to local inflammation and oxidative stress (13). Indoxyl

sulfate also seems to decrease macrophage cholesterol efflux (13), an important pathway for preventing foam cell formation. Human data support these mechanistic findings. Higher concentrations of circulating indoxyl sulfate and p-cresyl correlate with measures of vascular dysfunction (14,15) and aortic calcification (15), and they are independently associated with cardiovascular disease among individuals with kidney disease (14–17). However, not all studies agree with these findings. In a large cohort of 1273 patients on dialysis, indoxyl sulfate and p-cresol were not associated with cardiovascular events (18). Only in a subcohort of patients on dialysis with low serum albumin was p-cresyl significantly associated with cardiovascular events (18), suggesting that other factors may modify this relationship.

Trimethylamine-*N*-Oxide

Trimethylamine-*N*-oxide (TMAO) is a byproduct of bacterial metabolism of dietary choline and L-carnitine, and recent studies suggest that it directly promotes atherosclerosis (19–21). Intestinal bacteria metabolize dietary choline and L-carnitine to trimethylamine (19–21), which undergoes rapid intestinal absorption and subsequent oxidation in the liver by flavin mono-oxygenase enzymes to TMAO (22). Serum concentrations of TMAO increase with advancing CKD and are at least 30-fold higher in patients with ESKD (23). Data are conflicting as to whether these marked elevations result solely from decreased clearance by the kidneys or are due to increased production of this metabolite. Animal studies suggest that differences in microbiota diversity (24) may explain the significant interpatient variability of blood TMAO levels among patients with similar GFR (25). Recent human and animal data suggest that dysbiosis is directly responsible for higher TMAO levels observed in CKD (4). In these studies, patients with CKD showed a shift in the relative abundance of dominant intestinal bacteria compared with healthy controls; this shift in gut bacterial composition was accompanied by altered gene expression favoring trimethylamine production in patients with CKD. Moreover, when the gut flora from patients with CKD was used to repopulate the intestines of antibiotic-treated mice, serum TMAO levels increased (4).

In mice, TMAO directly induces atherosclerosis by increasing macrophage cholesterol scavenger receptors (19) and reducing reverse cholesterol transport (20), resulting in greater foam cell formation, a characteristic of early atherosclerosis. TMAO is independently associated with both prevalent and incident cardiovascular disease risk, including atherosclerosis, among non-CKD populations (19–21). Furthermore, higher circulating concentrations of TMAO are associated with all-cause mortality (23,26,27) and greater burden of coronary atherosclerosis (23) and ischemic cardiovascular events (25) among patients with CKD. However, among patients with ESKD, data are conflicting (28,29). Thus, if TMAO is a cardiovascular toxin, it seems that serum levels may better predict cardiovascular events among patients with preserved kidney function or moderate CKD (21,23,25).

Phenylacetylglutamine

Phenylacetylglutamine is a newly identified bacterial metabolite of amino acid fermentation. It is derived from the bacterial conversion of phenylalanine to phenylacetic acid, which is then conjugated with glutamine. Phenylacetylglutamine is absorbed into the circulation and efficiently cleared by the kidneys. Serum levels of this compound are elevated in advanced CKD (30). Although decrements in glomerular filtration may largely explain elevated serum concentrations of phenylacetylglutamine in CKD (30), a study evaluating 24-hour urinary excretion of phenylacetylglutamine among patients with predialysis CKD and patients with new kidney transplants suggests that microbiome changes may enhance the production and intestinal absorption of phenylacetylglutamine (31). Moderately sized observational studies suggest an independent relationship between higher serum phenylacetylglutamine levels and adverse outcomes among individuals with kidney disease. Among 488 patients with predialysis CKD, serum phenylacetylglutamine was independently associated with greater mortality and cardiovascular disease (31). Likewise, among 394 patients on hemodialysis, serum phenylacetylglutamine was also independently associated with cardiovascular disease events (32). However, in a larger cohort ($n=1273$) of patients on dialysis, there was no association with cardiovascular events (18). Because phenylacetylglutamine is a bacterial byproduct of amino acid metabolism and because there is some evidence that it may be influenced by the microbiome composition (32), it is a candidate for further investigation into the intersection of dysbiosis and cardiovascular disease in kidney disease.

Bile Acid Metabolism

Intestinal bacteria induce biotransformation of primary bile acids to secondary bile acids, including deoxycholic acid (DCA) (33). In CKD, although all circulating bile acids are elevated (34,35), the proportion of circulating DCA is increased compared with others (34). Thus, CKD-associated dysbiosis may be responsible for elevated circulating DCA levels. Bile acids, especially cheno-DCA, stimulate the farnesoid X nuclear receptor (FXR) to regulate lipid and glucose metabolism. The FXR may influence cardiovascular disease not only because dysregulation of lipid and glucose metabolism promotes endothelial dysfunction and atherosclerosis but also because FXR has been identified in atherosclerotic lesions and endothelial cells (36). Moreover, DCA is directly toxic to cultured vascular smooth muscle cells (35); thus, it may also have direct vascular toxicity. FXR activation decreases DCA levels and attenuates vascular toxicity by reducing vascular calcification (35,37) and atherosclerotic plaque formation in animal models (38). It is plausible that elevated circulating DCA levels along with disproportionately lower levels of other bile acids that have a higher affinity for FXR promote vascular calcification and atherosclerosis in CKD. We recently described an independent relationship between elevated circulating DCA levels and coronary artery calcification among patients with moderate CKD (39). Among 112 patients with a mean eGFR of 31.5 ± 8.7 ml/min per 1.73 m^2, those with a DCA level greater than the median of 58 ng/ml had significantly greater coronary artery calcification scores compared with those whose DCA level was less than or equal to the median. Likewise, when modeled as a continuous variable, higher DCA levels were associated with greater coronary artery calcification scores. These

relationships were unchanged and remained significant after adjustment for demographics, comorbidities, and markers of mineral metabolism, including calcium, phosphorus, parathyroid hormone, and fibroblast growth factor 23.

Potential Interventions

Modifying the intestinal microbiome may be a candidate therapy to mitigate cardiovascular disease among individuals with kidney disease. Therapies are aimed at changing the relative abundance of intestinal microbial species to improve inflammation and lower serum concentrations of bacteria-derived toxins, which are associated with cardiovascular disease. In recent small interventional trials, synbiotics (pre- and probiotics), antibiotics, sevelamer, and a high-fiber diet showed the ability to reduce serum indoxyl sulfate and p-cresol as well as change the composition of some intestinal microbial species among patients with CKD and patients with ESKD (Table 1). In animal models and small human trials, the oral adsorbent, AST-120, partially restored intestinal epithelial tight junction proteins, reduced inflammation and serum levels of indoxyl sulfate, and slowed kidney disease progression (3). However, a large randomized, placebo-controlled trial among 2035 subjects with CKD stage 4 showed no significant benefit in kidney disease progression or death of AST-120 (40). These conflicting results expose a potential gap in the understanding of the microbiome and its relationship to clinical outcomes, such as mortality and cardiovascular disease, in kidney disease. Interventions that shift the composition of the microbiome and even improve circulating concentrations of bacterial byproducts with purported organ toxicity do not necessarily translate to improved clinical outcomes. This conundrum illustrates the complexity of the microbiome and our ability to alter it to improve cardiovascular outcomes in kidney disease. More large interventional trials are needed to determine whether other therapies that modify the intestinal microbiome improve hard clinical end points in kidney disease.

Conclusions

Mounting evidence suggests that CKD is accompanied by shifts in the relative abundance of gut bacterial populations and defects in intestinal barrier function leading to systemic inflammation. The retention of metabolites derived from intestinal bacteria is a common observation in patients with CKD, possibly resulting from reduced clearance by the kidneys of these substances and alterations in their production and intestinal absorption. Translational research studies show that many bacterial byproducts directly stimulate pathways that promote deleterious changes in arteries. Numerous epidemiologic studies show a relationship between gut-derived vascular toxins and cardiovascular events in patients with CKD. Although various interventions may alter the composition of intestinal microbiota in CKD and ESKD, clinical benefit is not yet confirmed. As a result, the intestinal microbiome is emerging as a key target for research focused on understanding the contribution of nontraditional risk factors to cardiovascular morbidity in CKD. As an important first step to realizing the gut microbiome as a therapeutic target, efforts to better define microbiome changes in CKD are currently underway (NCT02572882 and NCT03265639).

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