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REVIEW

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Role of altered intestinal microbiota in systemic inflammation and cardiovascular disease in chronic kidney disease

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ABSTRACT: The normal intestinal microbiota plays a major role in the maintenance of health and disease prevention. In fact, the alteration of the intestinal microbiota has been shown to contribute to the pathogenesis of several pathological conditions, including obesity and insulin resistance, among others. Recent studies have revealed profound alterations of the gut microbial flora in patients and animals with chronic kidney disease (CKD). Alterations in the composition of the microbiome in CKD may contribute to the systemic inflammation and accumulation of gut-derived uremic toxins, which play a central role in the pathogenesis of accelerated cardiovascular disease and numerous other CKD-associated complications. This review is intended to provide a concise description of the potential role of the CKD-associated changes in the gut microbiome and its potential role the pathogenesis of inflammation and uremic toxicity. In addition, the potential efficacy of pre- and pro-biotics in the restoration of the microbiome is briefly described.

Background

Chronic kidney disease (CKD) patients have several risk factors for cardiovascular disease (CVD), and the rate of cardiovascular mortality is extremely high in these patients [1]. In addition to traditional risk factors (obesity, hypertension, diabetes and dyslipidemia, among others) and situations specific to kidney disease (hypervolemia, electrolyte disorders, anemia, changes in calcium-phosphorus metabolism and dyslipidemia), CKD patients have a high prevalence of so-called 'emerging risk factors', including hyperhomocysteinemia, increased lipoprotein(a), oxidative stress and inflammation [2].

Recent studies have identified intestinal microbiota imbalance [3–6] as a new factor that may contribute to inflammation and CVD. Indeed, the microbial inhabitants of the gut may affect the body's metabolic processes and should be considered an environmental factor that contributes to obesity and its comorbidities (insulin resistance, diabetes and cardiovascular disease). In this context, until recently, little was known about the gut microbiota in CKD [7].

In a recent study, Vaziri *et al.* [5] demonstrated that uremia profoundly alters the composition of the intestinal microbiota in humans and experimental animals. These changes may result in the production and absorption of noxious byproducts that can contribute to uremic toxicity, inflammation, malnutrition and other morbidities in uremic patients [8]. Moreover, the authors demonstrated that uremia results in the depletion of key protein constituents in the colonic epithelial tight junction

KEYWORDS:

- cardiovascular disease
- chronic kidney disease
- endotoxins • inflammation
- intestinal microbiota
- nutrients • oxidative stress
- prebiotic • probiotics
- uremic toxins

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in experimental animals, which is a phenomenon that can account for the impaired intestinal barrier structure and function in uremia [9]. Together, the alteration of the intestinal microbiome and impaired intestinal epithelial barrier structure and function can lead to endotoxemia and the accumulation of the gut-derived uremic toxin(s)/metabolite(s). This accumulation contributes to the metabolic perturbation, systemic inflammation and increased cardiovascular risk in CKD patients [7]. Therefore, the metabolic crosstalk between the microbiota and the development of cardiovascular disease appears to be a new challenge in the studies on CKD patients. This review provides a brief overview of the function of the microbiota and the possible role of its alteration in systemic inflammation and cardiovascular disease, with a particular emphasis on CKD patients.

Microbiota

The human gut contains approximately 10–100 trillion microorganisms and houses the largest and most diverse ecosystem of microbes in the human body, consisting of more than 400 species of bacteria [10]. Five bacterial phyla are present in the human gut (Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria and Verrucomicrobia), of which, two are dominant: Bacteroidetes (*Bacteroides*, *Prevotella* and *Xylanibacter*) and Firmicutes (*Ruminococcus*, *Clostridium*, *Lactobacillus*, *Eubacterium*, *Faecalibacterium* and *Roseburia*) [7].

The human microbiota plays an important role in the biochemical activities of the human body including the production of vitamins and other essential nutrients and the regulation of many aspects of immunity [10–12]. Through their constituent enzymes, the intestinal microbial flora participate in the fermentation of the ingested nutrients and the metabolism of bile acid. The latter leads to the conversion of the primary bile acids into secondary bile acids, which act as signaling molecules by binding to cellular receptors implicated in the modulation of lipids and glucose metabolism [13–15].

The composition of the diet can have a marked impact on the gut environment, including gut transit time, pH and available substrates that bacteria can use. Consequently, changes in the intakes of the three main macronutrients (proteins, lipids, and digestible and nondigestible complex carbohydrates) significantly affect the composition of the gut microbiota [15].

Carbohydrates

Nondigestible carbohydrates are important sources of energy for several members of the colonic microbiota. Out of the short-chain fatty acids (SCFAs) produced by the microbial fermentation of nondigestible carbohydrates, butyrate is particularly important as a source of energy for the colonic epithelium and the acetate and propionate produced by microbial flora are used as substrates for lipogenesis and gluconeogenesis by the liver. Moreover, SCFAs can regulate gene expression, suppress inflammation and modulate GLP-1 secretion, improving insulin secretion and exerting antidiabetic effects [16–19].

In addition, SCFAs activate a free fatty acid receptor (FFAR), which is a G-protein-coupled receptor (GPCR). Acetate and propionate seem to activate FFAR2 and butyrate activates FFAR3, which play important roles as physiological sensors for food-derived free fatty acids and digestion products in the GI tract. Moreover, they are involved in secretion of insulin and incretin hormones, and regulation of the sympathetic nervous systems, taste preferences and inflammatory responses caused by insulin resistance [20,21]. According to Pluznick *et al.* SCFAs are also involved in blood pressure control through the modulation of specific receptors (i.e., Olfr78 and Gpr41) [22].

Fats

The available data regarding the effects of dietary fat on human microbiota are limited; however, some studies have demonstrated a link between high-fat diet, gut inflammation and high levels of circulating lipopolysaccharide (LPS) [23,24]. Moreover, fat composition can modulate systemic inflammation and endotoxemia. Laugerette *et al.* demonstrated that a high-fat diet containing palm oils, as opposed to rapeseed or sunflower oil, results in a greater increase in inflammation markers in the plasma and adipose tissue and circulating LPS [24].

Proteins

The main pathway of amino acid fermentation in the human colon is deamination, which leads to the production of SCFA and ammonia. Most of the ammonia produced is rapidly absorbed, converted into urea by the liver, and excreted in the urine. Moreover, the bacterial deamination of aromatic amino acids leads to the production of the phenolic compounds. For instance, tyrosine mainly yields phenol and

p-cresol, while phenylalanine yields phenylacetate and tryptophan yields indoleacetate and indole. The latter is metabolized in the liver, producing indoxyl sulfate. During its transport through the intestinal epithelial cells, *p*-cresol is converted to *p*-cresyl sulfate by cytoplasmic sulfotransferase and released in the circulation. More than 90% of urinary phenolic compounds are present as *p*-cresyl sulfate, which is excreted by the kidney, mainly through proximal tubular secretion [25]. The bacteria involved in this conversion include species within the *Clostridium*, *Bacteroides*, *Enterobacterium*, *Bifidobacterium* and *Lactobacillus* families [15].

Methionine and cysteine (sulfur-containing amino acids) can produce hydrogen sulfide (H₂S) when fermented by microbiota and, at high levels, can inhibit colonic epithelial cell respiration and cause DNA damage [26]. The decarboxylation of amino acids and peptides also leads to the formation of various amines that can act as precursors in nitrosamine formation [27].

Another nutrient metabolized by the microbiota is phosphatidylcholine, also known as lecithin, which is found in eggs and red meat. The gut microbiota releases choline from dietary phosphatidylcholine, where it is then metabolized into toxic trimethylamine (TMA) [27]. In the liver, TMA is converted by flavin-containing monooxygenases to trimethylamine-*N*-oxide (TMAO), which can lead to nonalcoholic fatty liver. Furthermore, the plasma levels of TMAO and its metabolites are correlated with the risk of CVD [28–30].

The carbohydrate/nitrogen ratio and the nature of the carbon catabolites have a profound impact on the metabolism and byproducts of the microbiota. Heavy influx of nitrogenous waste products (urea and uric acid) into the intestinal tract, together with dietary restriction of undigestible complex carbohydrates in CKD patients significantly alters the carbohydrate/nitrogen ratio. These changes can, in turn, modify the metabolism of intestinal microbiota via the catabolite repression control pathways and other adaptive mechanisms.

In summary, the metabolic role of the normal intestinal microbiota is essential for the normal biochemical activities of the human body. However, changes in the composition and function of the microbiota can lead to serious adverse consequences due to production of toxic and harmful products, such as toxic gases, indoxyl sulfate, *p*-cresyl sulfate, amines, ammonia and

TMAO. In addition, under pathological conditions, lipopolysaccharides (LPS) present in the outer membrane of gram-negative bacteria can be absorbed into the bloodstream, causing systemic inflammation that, if persistent, can lead to CVD and various other morbidities [27,31].

Role of the altered intestinal microbiota in the pathogenesis of systemic inflammation & cardiovascular disease

The Firmicutes/Bacteroidetes ratio is an important factor in the composition of the intestinal microbiota and may change significantly with the use of antibiotics, certain dietary nutrients and pathological conditions [4,32,33]. Recently, authors have observed that, during pregnancy, remodeling of the gut microbiota occurs, with enrichment of proteobacteria and actinobacteria during the third trimester. This remodeling may potentially modulate the immune system and facilitate metabolic and immunological adaptations [34,35].

Host remodeling of the gut microbiome & metabolic changes during pregnancy

Recent work suggests that altered gut microbiota can be associated with metabolic diseases; for example, obese people seem to have fewer Bacteroidetes and more Firmicutes than lean individuals [32,36]. Likewise, the reduction in the Bacteroidetes/Firmicutes ratio increases susceptibility to infections, immune disorders, inflammation, oxidative stress and insulin resistance, events that are mediated by so-called ‘metabolic endotoxemia’, which involves exposure to noxious intestinal products, particularly LPS [31,37]. Several factors including chronic kidney disease, excessive alcohol consumption, stress, exposure to radiation, a high-fat diet, and the composition of fat have been shown to increase plasma endotoxins in the absence of detectable infection [37,38].

In addition, dysregulation of the peripheral and adipose tissue endocannabinoid system (ECS) contributes to increased plasma concentrations of LPS [39]. The endocannabinoids are derivatives of polyunsaturated fatty acids, among which anandamide (*N*-arachidonoyl ethanolamine) and 2-arachidonoyl glycerol (2-AG) have been well studied. The ECS is a signaling system with important neuromodulatory functions that is involved in physiological processes, such as the modulation of synaptic transmission, regulation of immune function,

inflammatory and antiproliferative action against tumor cells, control of the cardiovascular system, and increased appetite and body weight [40]. Despite its benefits, this system can increase the intestinal epithelial barrier permeability, thus affecting the entry of LPS into systemic circulation [39,40].

A brief description of the role of some of the microbial structural components and metabolic byproducts, as well as their involvement in the pathogenesis of inflammation and cardiovascular disease in general and of CKD in particular is provided here.

Lipopolysaccharides

LPS is a component of the cell wall of the Gram-negative bacteria. Exposure to this endotoxin initiates an inflammatory response and oxidative stress by binding to Toll-like receptor-4 (TLR4) on endothelial cells and monocytes/macrophages, leading to the activation of NF-κB and AP-1. This results in the production of pro-inflammatory cytokines, chemokines, adhesion molecules and reactive oxygen species (ROS), which can cause endothelial damage and dysfunction, and promote atherosclerosis [41,42]. Production of ROS by these cells is mediated by activation of NAD(P)H oxidase type II (NOX-2), which involves assembly of its cytoplasmic and membrane-bound subunits. Interestingly, genetic mutations of the different subunits of NOX-2 have been linked to the pathogenesis of diverse disorders including chronic granulomatous disease [43], myelodysplasia [44] and a form of colitis resembling Crohn's disease [45]. Accordingly the host's genotype plays an important role in its response to the changes in the gut microbiome.

• Harmful microbial metabolites

In addition to the bacterial components, such as lipopolysaccharides, that induce inflammation, certain bacterial metabolites can exert cytotoxicity and promote inflammation, tissue injury and dysfunction. For instance, TMAO has been shown to contribute to the development and progression of atherosclerosis and cardiovascular disease [46]. Wang *et al.* demonstrated the critical role of dietary choline and gut microbiota in TMAO production, macrophage cholesterol accumulation and foam cell formation in rats [30]. In fact, Lewis *et al.* identified the value of metabolic profiling, including TMAO, in the early detection of myocardial injury [47].

One of the most widely studied metabolites from the microbiota is *p*-cresyl sulfate, which has been shown to readily penetrate the endothelial cell membrane and cause endothelial damage and dysfunction. *In vitro*, *p*-cresyl sulfate induces the shedding of the endothelial microparticles and disrupts nitric oxide signaling, events that support the observed association of the elevated plasma levels with the risk of cardiovascular disease [48,49]. Moreover, administration of *p*-cresyl sulfate in normal rats for 4 weeks has been shown to cause insulin resistance and thus represents a major risk factor for cardiovascular disease [50].

The other widely studied toxin generated by the gut microbiota is indoxyl sulfate, which has been shown to stimulate vascular smooth muscular cell proliferation, NAD(P)H oxidase activation, ROS production and endothelial senescence, and can impair endothelial healing [51-53]. The increased production and impaired renal excretion of *p*-cresyl sulfate and indoxyl sulfate results in their accumulation in patients with renal failure. The accumulation of these products has been shown to cause vascular dysfunction in humans and experimental animals with CKD [48-53].

• Urea-derived ammonia & ammonium hydroxide

Uremia results in the impairment of the intestinal epithelial barrier structure and function, which plays a major part in the pathogenesis of systemic inflammation [7,54]. As described below, emerging evidence points to the role of urea as a major mediator of the intestinal epithelial barrier dysfunction and resultant systemic inflammation. The accumulation of urea in the body fluids of humans and animals with renal failure leads to its heavy influx into the GI tract. Within the intestinal tract, urea is hydrolyzed by microbial urease to form large quantities of ammonia [$\text{CO}(\text{NH}_2)_2 + \text{H}_2\text{O} \rightarrow \text{CO}_2 + 2\text{NH}_3$], which is then converted to ammonium hydroxide [$\text{NH}_3 + \text{H}_2\text{O} \rightarrow \text{NH}_4\text{OH}$]. As described below, ammonia and ammonium hydroxide have recently been shown to interact with and disrupt the protein constituents of the intestinal epithelial tight junctions. This phenomenon leads to subclinical enterocolitis and systemic inflammation by enabling the entry of endotoxins and other noxious luminal contents into the underlying tissues and systemic circulation. The heavy influx of urea in the GI

tract is compounded by the colonization of the upper intestinal tract and the dramatic change in the composition of the gut microbiome in the uremic humans and animals [5]. Recent *in vitro* studies using cultured human colonocytes lend support to the role of urea-derived ammonia. These studies have demonstrated that adding clinically relevant concentrations of urea to the culture media caused concentration-dependent falls in the transepithelial electrical resistance and depletion of the tight junction proteins in cultured human colonocytes. The addition of urea plus urease, which is designed to simulate the presence of bacteria in the gut, leads to the complete loss of transepithelial electrical resistance and the near total loss of the tight junction proteins [55]. These studies identified urea and urea-derived ammonia as the main mediators by which exposure to uremic patients' plasma caused the depletion of epithelial tight junction proteins and the impairment of the barrier function in cultures of human colonocytes, as demonstrated in an earlier study [56]. The latter study revealed a marked impairment of the epithelial barrier structure and function in cultured colonocytes that were exposed to the pre-hemodialysis plasma as well as lesser effects from the post-hemodialysis plasma from CKD patients. The role of urea-derived ammonia was supported by a recent study that revealed a significant improvement in the epithelial tight junctions and an attenuation of the systemic oxidative stress, inflammation and endotoxemia in CKD rats that had been treated with oral activated charcoal [57]. The ammonia generated from the hydrolysis of urea by the intestinal microbial flora is absorbed and converted to urea by the liver. The blood urea concentration is significantly lower in activated charcoal-treated rats than in untreated CKD rats, denoting the adsorption and removal of ammonia by activated charcoal. Together, these experiments have helped to elucidate the role of urea (which until recently was considered to be a harmless uremic retained metabolite) as a major mediator of systemic inflammation, and the associated cardiovascular and numerous other complications in the CKD population [57].

• Intestinal microbiota & inflammation & CVD in CKD patients

In healthy individuals, the toxins produced by intestinal bacteria (indoxyl sulfate, *p*-cresyl sulfate, amines, ammonia and TMAO) are

eliminated by the kidneys. However, in CKD patients, they accumulate in body fluids. Owing to their protein-binding properties, some of these toxins cannot be efficiently removed by dialysis modalities. These compounds are called uremic toxins because they are cytotoxic, negatively impact the biological functions, and exert pathological effects on the cardiovascular and immune systems [3,58–61]. In addition, as noted above, the uremia-induced disruption of the intestinal epithelial barrier structure enables the influx of endotoxins into the systemic circulation. Via the activation of NF- κ B and AP-1, which are master regulators of the inflammatory cytokines, chemokines, and profibrotic pathways, CKD-associated endotoxemia promotes systemic inflammation, oxidative stress, cardiovascular and numerous other disorders [62]. Indeed, some studies have shown a positive correlation between LPS from the intestinal microbiota and C-reactive proteins in patients on peritoneal dialysis and hemodialysis [62,63]. In fact, circulating monocytes and neutrophilic granulocytes in ESRD patients exhibit the marked upregulation of endotoxin receptor (TLR4) and increased production of superoxide and hydrogen peroxide in a resting state [64,65]. The ongoing activation of leukocytes leads to their dysfunction and impairs their phagocytic capacity [66].

Indoxyl sulfate, a byproduct of the intestinal microbial flora, has emerged as a potent uremic toxin and has been shown to promote the progression of renal and cardiovascular disease by inducing oxidative stress, inflammation and fibrosis. Recent studies have shown that serum indoxyl sulfate levels are a reliable marker in predicting CVD, and that indoxyl sulfate and *p*-cresyl sulfate are reliable CKD progression markers in CKD patients [61,67,68].

p-cresyl sulfate has also been found to be associated with the occurrence of CVD and elevated mortality in CKD patients [4,69,70]. Elevated plasma levels of *p*-cresyl sulfate are associated with the high concentrations of endothelial microparticles in ESRD patients [50]. These microparticles are related to increased arterial stiffness and act as pro-thrombotic and pro-inflammatory mediators [71]. The role of *p*-cresyl sulfate in mediating the release of the endothelial microparticles was confirmed by *in vitro* studies using cultured human endothelial cells [50]. It is of interest that the association between plasma *p*-cresyl sulfate levels and the risk of cardiovascular disease is not

limited to the CKD population and can also be found among patients with no renal diseases [72].

In relation to the TMAO levels in CKD patients, few studies have been published, and none have explored its role in CVD in these patients. Bain *et al.* observed high TMAO levels before hemodialysis and their efficient removal by hemodialysis [73].

The imbalance of the intestinal microbiota seems to be involved in the above mentioned abnormalities. In a recent review, Anders *et al.* discussed the activation of innate immunity by bacterial products, which could cause systemic inflammation in CKD patients [74]. Vaziri *et al.* showed that gut microbiota is altered in uremia [5]. However, the biological impact of this phenomenon is presently unknown and requires further investigation. Preliminary data from our study in Brazil confirmed an altered composition of the gut microbiota in 12 patients with stage III–IV CKD when compared with healthy individuals. A reduced fecal microbial diversity was observed upon the PCR-denaturing gradient gel electrophoresis of 16S rRNA gene fragments (this method involves a molecular sequence-dependent fingerprinting technique that allows the characterization of the intestinal microbiota without pre-existing knowledge of its composition) in CKD patients (30.7 ± 5.2 bands) when compared with healthy individuals (42.8 ± 3.4 bands) (Figure 1).

Manipulating the microbiota using pre/probiotics may be a promising strategy to attenuate

the imbalance in the microbiota and has been suggested as a potential intervention to minimize the adverse effects caused by the accumulation of toxic substances generated by the intestinal microbiota in CKD.

Effects of probiotics & prebiotics in CKD

By modulating the composition of the intestinal microbiota, the use of probiotics could potentially minimize the deleterious effects of its imbalance, thereby improving the health of the gastrointestinal tract, strengthening the immune system, restoring the bioavailability of micronutrients, exerting antidiabetic actions, improving dyslipidemia and allergic disorders, and reducing the risk of certain cancers [75].

The mechanism by which probiotics exert their favorable effects seems to include changes in intestinal pH, suppression of pathogens (through the production of antibacterial compounds, competitive exclusion of pathogens in receptor binding sites and competition for available nutrients), suppression of mutagenic and carcinogenic processes and protection of the intestinal barrier [76,77].

Previous studies have demonstrated the immune modulatory and anti-inflammatory effects of probiotics, leading to a reduction in the influx of LPS into the systemic circulation, the attenuation of the TLR-4 mediated activation of NF-κB, the formation of proinflammatory cytokines, reductions in systolic blood pressure and fibrinogen levels, and interactions with immune cells, leading to improvements in

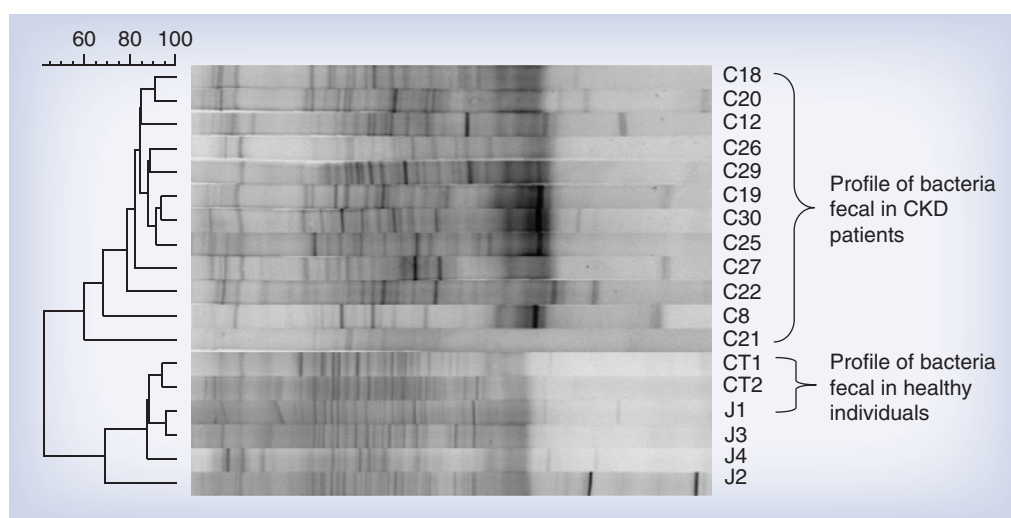


Figure 1. Denaturing gradient gel electrophoresis fingerprints from healthy individuals and chronic kidney disease patients.

CKD: Chronic kidney disease.

intestinal integrity [78,79]. In addition, the use of probiotics may confer cardioprotective effects, including the prevention and attenuation of ischemic heart disease and the reduction of serum cholesterol [80].

Regarding kidney disease, Simenhoff *et al.* were the first to administer *lactobacillus* to hemodialysis patients and to observe reductions in dimethylamine (DMA) and nitrosodimethylamine, which are toxins produced by the small intestine [81]. Other researchers have shown reductions in indoxyl sulfate, homocysteine and triglycerides levels with the oral supplementation of bifidobacteria in hemodialysis patients [82,83]. Ranganathan *et al.* also reported the prolongation of life, reduction in blood urea concentration and decreased progression of renal disease with 16 weeks of probiotic supplementation in uremic rats [84]. In a pilot study, the same group [85] found improvements in the symptoms and quality of life in patients with stages III and IV CKD who received 6 months of supplementation with probiotics.

Notably, the driving force behind changes in the intestinal microbiome is the uremic milieu. Consequently, the introduction of probiotics may be ineffective or less effective, as their survival may be hampered by the uremic environment of the gut into which they are introduced.

Another strategy for modulating the intestinal microbiota is the use of prebiotics, such as oligofructose inulin polymers, fructooligosaccharides, galactooligosaccharides and other oligosaccharides. Prebiotics have proven effective in improving glycemic control and plasma lipid profiles. The mechanisms of action of prebiotics are complex, and several hypotheses have been proposed in the literature, including: soluble fibers may reduce the digestion of macronutrients by delaying gastric emptying and/or intestinal transit time; improve the secretion of GLP-1; increase the production of SFAs; modulate the composition of colonic microbiota, reducing LPS and increasing the contents of gut bifidobacteria [86].

Several studies have shown reductions in urinary *p*-cresyl sulfate and indoxyl sulfate levels with the use of prebiotics, such as oligo-fructose, in healthy subjects, as well as in hemodialysis patients [59,87]. Moreover, in CKD mice, treatment with prebiotics has been shown to reduce the production of *p*-cresol and to improve insulin resistance and dyslipidemia. Finally, we have observed reductions in the blood urea level with the administration of a prebiotic diet

for 3 months in CKD mice [KOPPE L, UNPUBLISHED DATA]. The effect of prebiotics on other intestinal metabolites in CKD is currently unknown, and studies on the efficacy of prebiotics in preventing CKD-associated metabolic disturbances in humans are lacking (Figure 2). Nakabayashi *et al.* used supplemental symbiotics (probiotic combined with prebiotic) for two weeks and observed significant reductions in *p*-cresyl sulfate levels and the normalization of bowel habits in hemodialysis patients [88]. Furthermore, AST-120, an oral adsorbent capable of binding solutes and preventing their intestinal absorption represents a type of therapy able to reduce the levels of indoxyl sulfate [89]. In addition, a recent study demonstrated that supplementation with AST 120 appears to improve intestinal barrier, inflammation and oxidative stress of CKD [90].

More recently, fecal microbiota transplantation has been suggested as a new approach to restore the dysbiosed microbiota. However, experience with this technique is limited and requires careful consideration [91,92]. In addition, the development of genetically modified probiotics may be beneficial; for example, clinical benefit was observed with genetically modified *L. lactis* treatment to deliver IL-10 from intestinal mucosa in Crohn's disease patients. Although engineering probiotics is promising, their safety must be further validated [93].

Conclusion

In conclusion, the gut microbiota plays an important role in health, and changes in its composition can contribute to systemic inflammation and the generation of uremic toxins, both of which have been linked to cardiovascular mortality in dialysis patients. The potential efficacy of pro- or pre-biotics in attenuating CKD-associated inflammation and CVD in humans with CKD is unknown and awaits future investigation.

Future perspective

It is well known that the leading cause of mortality in CKD patients is cardiovascular disease. The imbalance in the composition of the intestinal microbiota in these patients may represent an emerging risk factor because this imbalance can contribute to an increase in chronic inflammation and oxidative stress. Accordingly, interventions aiming to restore and maintain the balance and function of the intestinal microbiota are extremely important.

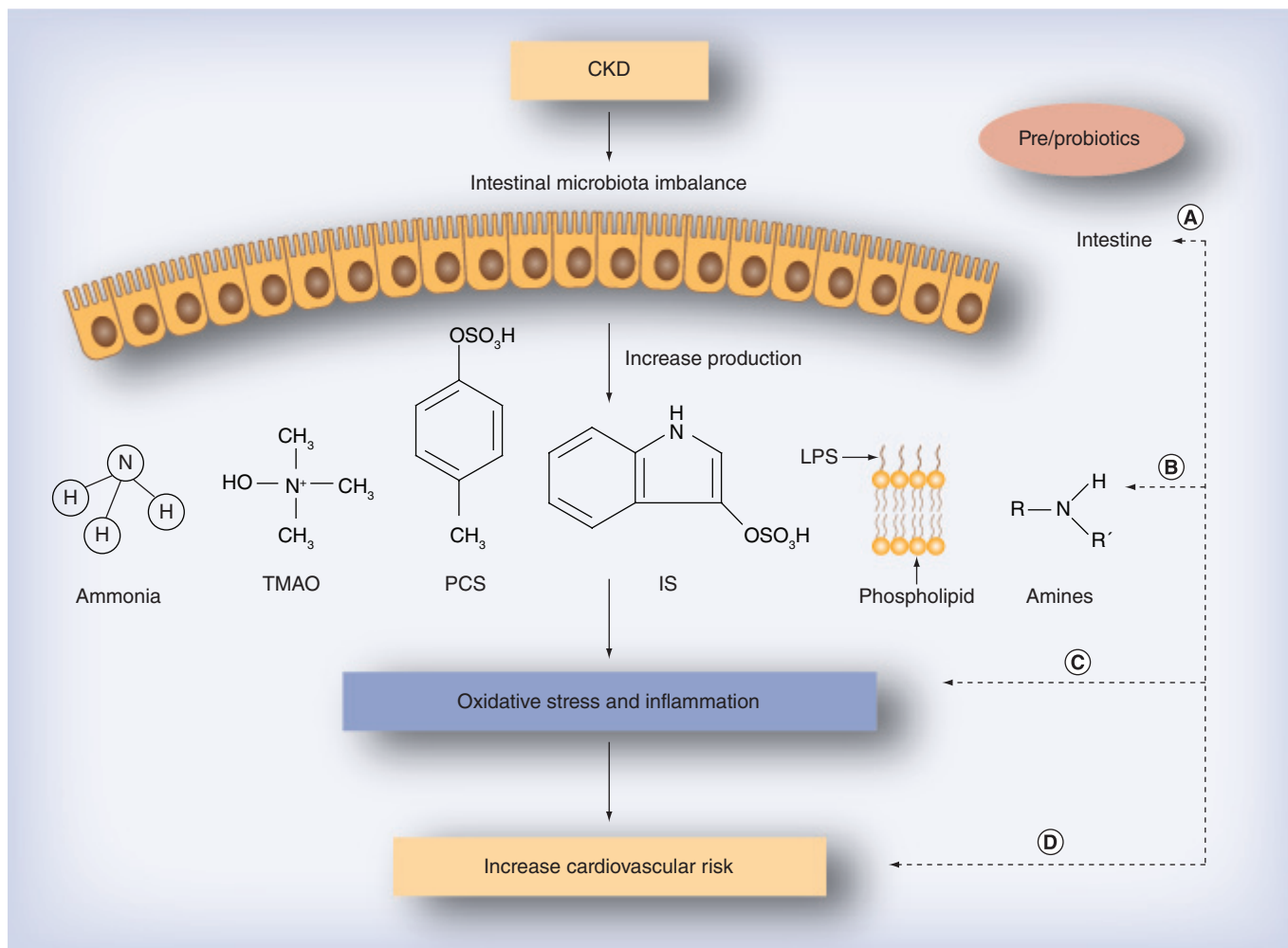


Figure 2. The possible effects of pre/probiotics on intestinal metabolites in chronic kidney disease patients. CKD leads to an intestinal microbiota imbalance and patients accumulate the toxins produced by intestinal bacteria (IS, PCS, amines, ammonia and TMAO) in plasma. These compounds interact negatively with biological functions, resulting in pathological impact such as increased oxidative stress and inflammation, increasing the cardiovascular risk. The use of pre- or pro-biotics can modulate the intestinal microbiota minimizing the deleterious effects caused by its imbalance and thereby promotes improved health of the gastrointestinal tract (A), reducing plasma levels of intestinal toxins (B), reducing oxidative stress and inflammation (C), and attenuating cardiovascular risk in CKD patients (D).

CKD: Chronic kidney disease; IS: Indoxyl sulfate; LPS: Lipopolysaccharides; PCS: *p*-cresyl sulfate; TMAO: Trimethylamine-*N*-oxide.

Preliminary studies by Ranganathan's group have demonstrated the beneficial effects of probiotic supplementation in renal patients [76,77]. However, these studies did not assess any markers of inflammation and oxidative stress. In addition, the sample was small, therefore further studies are required to confirm the efficacy of this intervention.

In the future, we may be able to verify whether alterations in the composition of the microbial community in CKD patients constitute a new cardiovascular risk factor and whether the composition of the intestinal

microbiota in CKD patients is influenced by different treatment modalities. In addition, we may be able to determine whether a relationship exists between the composition of the intestinal microbiota and the presence of inflammation, oxidative stress and nutritional status. Finally, we should explore the effects of probiotic supplementation on markers of inflammation and oxidative stress.

Author contribution

D Mafra, JC Lobo and AF Barros were responsible for the conception, design and interpretation of data and

reviewed the article for critically important intellectual content. D Fouque and ND Vaziri reviewed the article for critically important intellectual content.

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EXECUTIVE SUMMARY

Microbiota

- The human gut microbiota is dominated by five bacterial phyla (Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria and Verrucomicrobia) and one Archaea (Euryarchaeota) and the diet composition can have a marked impact on the gut environment, including gut transit time, pH and available substrate for use by bacteria.

Role of altered intestinal microbiota in the pathogenesis of systemic inflammation & cardiovascular disease in chronic kidney disease patients

- The firmicutes/bacteroidetes ratio is an important factor in the composition of the intestinal microbiota and may change dramatically under certain pathological conditions.
- Uremia results in the impairment of the intestinal epithelial barrier structure and function, which plays a major role in the pathogenesis of systemic inflammation, associated cardiovascular disease and various other complications.
- Exposure to endotoxins (lipopolysaccharides), and uremic toxins (trimethylamine-*N*-oxide, *p*-cresyl sulfate and indoxyl sulfate) result in the production of proinflammatory cytokines, chemokines, adhesion molecules and reactive oxygen species, which can promote atherosclerosis.

Use of probiotics & prebiotics in chronic kidney disease

- Potential efficacy of pro/prebiotics in attenuating chronic kidney disease-associated inflammation and cardiovascular disease in humans with chronic kidney disease is unknown and awaits future investigation

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