Intestinal microbiota and diabetic kidney diseases: the Role of microbiota and derived metabolites in modulation of renal inflammation and disease progression

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Diabetic kidney disease (DKD) represents a growing public health burden and is the leading cause of end-stage kidney diseases. In recent years, host-gut microbiota interactions have emerged as an integral part for host homeostasis. In the context of nephropathies, mounting evidence supports a bidirectional microbiota-kidney crosstalk, which becomes particularly manifest during progressive kidney dysfunction. Indeed, in chronic kidney disease (CKD), the “healthy” microbiota structure is disrupted and intestinal microbes produce large quantities of uremic solutes responsible for renal damage; on the other hand, the uremic state, fueled by reduced renal clearance, causes shifts in microbial metabolism and composition, hence creating a vicious cycle in which dysbiosis and renal dysfunction are progressively worsened. In this review, we will summarize the evidence from clinical/experimental studies...
Introduction: the “gut-kidney axis” in diabetic kidney diseases

The worldwide prevalence of type 1 diabetes (T1D) and type 2 diabetes (T2D) has considerably increased in the last decades accounting for an enormous clinical burden due to the consequential rise in the prevalence of micro- and macro-vascular complications [1]. It is estimated than 30–40% of diabetic patients develop diabetic kidney disease (DKD), which is the most common microvascular complication and the primary cause of end-stage kidney disease (ESKD) [2]. Conventional treatment of DKD with renin-angiotensin-aldosterone system inhibitors (such as angiotensin II receptor blockers and angiotensin-converting enzyme inhibitors), along with novel glucose-lowering drugs, such as sodium-glucose transporter 2 inhibitors, have been proven beneficial in reducing diabetes-related cardiovascular morbidity and slowing the progression of CKD in T2D patients [3–13]. However, the residual risk of progression to ESKD remains high, urging the development of new therapeutic targets and approaches to promptly treat DKD or prevent its insurgence. Modulation of kidney inflammation and the gut-kidney axis may represent promising therapeutic targets to harness the progression of DKD to ESKD, for which the solely effective treatment is renal transplantation [14].

The intestinal tract, particularly the large intestine, harbors trillions of microbes, which live in a symbiotic relationship with the host. While the host ensures an anaerobic habitat and nutrition to the microbiome, the indigenous microbes provide the host with important metabolites through the bacterial synthesis of vitamins, secondary bile acids and metabolism of dietary proteins and carbohydrates. Accumulating evidence have now shown that a balanced symbiotic gut-host relationship is closely related to host health, and a disequilibrium in gut microbial communities and microbiota functions (dysbiosis) may be a central player in the pathophysiology of intestinal and extra-intestinal disorders, including diabetes and kidney diseases [15]. Indeed, observational studies in humans have reported that adiposity and impaired glucose metabolism are associated with low bacterial richness and compositional changes in the intestinal microbiota of individuals with T2D and obesity [16–18]. Experimental models and clinical trials have further shown the mechanistic involvement of the microbiome in host metabolism and metabolic disease pathogenesis by proving that the “lean/obese” phenotypes can be transmitted in part via fecal microbiota transplantation [19–22].

The intertwined bidirectional relationship between intestinal microbiome and kidneys (coined the gut-kidney axis) becomes particularly relevant during manifest chronic kidney disease (CKD). In fact, upon CKD, uremic toxins of microbial origin build up in the circulation (due to the loss of renal clearance) triggering a sequelae of inflammatory, apoptotic and fibrotic pathways, which perpetuate the progression of renal pathology [23] (Fig. 1).

In this review, we will summarize the current knowledge on the taxonomic and functional features of the gut microbiota in CKD and DKD; furthermore, we will discuss the latest evidence on the implication of microbial metabolic products in governing injurious and inflammatory processes in the diabetic kidney. Lastly, we will discuss the potential future directions in the “gut-kidney” research field and what constitute promising microbiome-targeting therapies against DKD.

DKD as a metabolic-driven immunological disease

Hyperglycemia, vascular damage and inflammation are important pathological components in DKD-associated renal injury and dysfunction [24]. Importantly, the pathological alterations of DKD are
similar between individuals with T1D and T2D, suggesting common underlying processes in hyperglycemia-associated renal dysfunction. The structural changes in renal morphology, however, are more heterogeneous in T2D, likely due to different sources of damage, such as obesity, hypertension and diabetes [25]. The diabetes-associated hemodynamic and metabolic disturbances progressively lead to glomerular hypertrophy, mesangial matrix expansion, loss of fenestrated endothelium, and injury to podocytes. These early changes to the glomerular filtration barrier are accompanied by nodular sclerosis, glomerulosclerosis, immune cell infiltrates and, lastly, tubulointerstitial fibrosis, a hallmark feature of all CKD. Albuminuria together with progressive decline in estimated glomerular filtration rate (eGFR) constitutes the prototypical clinical marker in predicting progression of DKD to ESKD.

Although generally not regarded as an immune-mediated disease, several inflammatory immune components instigate and support the development of DKD. Hyperglycemia results in the secondary generation of toxic advanced glycation end-products (AGEs) and reactive oxygen species (ROS), which are main drivers in the pathophysiology of DKD by inducing intracellular oxidative stress and activation of the proinflammatory nuclear factor-κB (NF-κB) [3,26–28]. NF-κB is considered the main transcription factor in the initiation of inflammatory responses in DKD. Not surprisingly, its activation has been documented in both human and experimental models of DKD and its inhibition was shown to ameliorate renal inflammation, oxidative stress, structural lesions and albuminuria in experimental DKD models [29–33]. In line, strategies aiming at blocking the functional signaling of AGEs or of downstream cytokines have shown promising results in ameliorating clinical and histological parameters of DKD [34–37].

With respect to the role of immune cells, macrophage influx in kidneys is critical in the progression of diabetic renal injury; macrophages represent the majority of renal infiltrating cells in DKD and their
influx rate correlates with the degree of proteinuria, glomerular and tubular damage, and fibrosis [38–42]. Macrophages may drive kidney damage by attracting other immune cells and by driving pro-inflammatory cytokine production.

Although not as prominent as macrophages influx, T lymphocytes traffic to diabetic kidneys in response to the renal upregulation of chemokines and adhesion molecules [29,38,43–47]. Similarly to macrophages, T lymphocytes exacerbate diabetes-related complications by producing pro-inflammatory cytokines, such as interferon-γ (IFN-γ) [48–50]. The latter is a typical CD4 T helper 1 (Th1) cytokine that promotes cell-mediated immunity and macrophage activation and elevated concentration of IFN-γ have been found in circulation and urine of DKD patients when compared to individuals with diabetes without overt nephropathy [49,51]. Another important subset of CD4+ T cells for DKD comprises regulatory T cells (Treg), as they hamper immune responses. In DKD patients, the frequency of Treg in peripheral blood is lower than in healthy controls and associate with DKD course; instead adoptive transfer of Treg in diabetic mice ameliorates diabetic nephropathy [52,53]. Overall, more studies need to address the exact function of distinct T lymphocytes subsets in DKD.

In addition to the influx of innate and adaptive immune cells to damaged kidneys, a critical role for pathogen recognition receptors (PRRs) in the pathogenesis of DKD has emerged in recent years. These innate immune receptors are expressed by infiltrating leukocytes as well as intrinsic kidney cells and can sense both pathogenic and endogenous damage signals, thereby initiating an inflammatory response to a wide array of ligands [54]. Notably, Toll-like receptors (TLR) can respond to microbial components as well as to endogenous molecules, such as metabolites elevated in obesity and diabetes (e.g. oxidized low-density lipoprotein, AGEs and non-esterified free acids [NEFA]), cellular stress/injury-related molecules, such as high mobility group box protein 1 (HMGB1) and heat-shock proteins (HSPs), as well as extracellular matrix components, such as fibrinogen, hyaluronan, biglycan and heparan sulfate [54]. In kidneys, TLRs, namely TLR2 and TLR4, are expressed in different renal compartments, including proximal tubules, glomerular endothelium, podocytes and mesangial cells, and are key contributors to renal injury [55–57]. Indeed, TLR4 was shown to be markedly elevated in renal glomeruli and tubules of T2D patients with microalbuminuria and overt DKD. Moreover, glomerular TLR4 expression associated with subsequent kidney dysfunction in a 6-year follow-up study of microalbuminuric patients [58]. In addition, several in vivo investigations using TLR4 genetic deficiency, neutralizing antibodies or signaling inhibitor have demonstrated the participation of TLR4 activation in the perpetuation of renal inflammation and promotion of renal injury, dysfunction and fibrosis in diabetic kidneys [55,59–61]. In a similar manner, experimental evidence has proven a role for TLR2 in prompting inflammation, renal injury and hence DKD progression. Indeed, in animal models TLR2 expression was enhanced in renal tubules and macrophages; whereas its deficiency abrogated the proinflammatory state, podocyte loss and microalbuminuria [56,62].

Similar to TLRs, nucleotide-binding oligomerization domain (NOD)-like receptors (NLR) can sense microbial and endogenous stress signals and initiate pro-inflammatory reactions. Particularly, the NLRP3 inflammasome complex, constituted by NLRP3, the adaptor ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain) and the effector pro-caspase-1, drives the production of active interleukin (IL)-1β and IL-18 and has been implicated in the pathogenesis of DKD [63,64]. The inflammasome activation, within podocytes, endothelial and tubular epithelial cells, has been demonstrated upon hyperglycemia, obesity and lipotoxicity, and appears to be largely driven by mitochondrial ROS production and mitochondrial dysfunction, typical hallmarks of CKD and DKD [65–70]. Indeed, the renal expression of NLRP3 inflammasome activation markers has been associated to the severity of albuminuria in CKD patients. Importantly, for a therapeutic prospective, administration of the NLRP3 inhibitor MCC950 ameliorated urinary ACR (urine albumin to creatinine ratio) and alleviated podocytes injury and renal fibrosis [71].

Overall, in DKD, TLRs sense microbial and endogenous danger-molecules generated during diabetes activating the NF-κB pathway while NLRP3 responds to intracellular metabolic stress and instigates pro-inflammatory cascades via IL-1β and IL-18. The triggers of this inflammatory response have not been completely elucidated in DKD; however, as described later in this review, PRR activation has been linked to alterations in gut microbiota composition and variations in circulating levels of microbially-produced metabolites.
Microbiota modulation of host immunity

The intestinal microbiota harbors trillions of bacteria, archaea, viruses and fungi. It is estimated that the number of bacteria inhabiting our gut outnumbers the number of cells in the human body with Firmicutes and Bacteroides being the dominant bacterial species [72]. Hence, it is not surprising that it is proposed by some that the gut microbiome is our largest exteriorized endocrine organ. The gut microbiome actively participates in host physiology and homeostasis by processing inaccessible nutrients from the diet, synthesizing vitamins, neurotransmitters and secondary bile acids and promoting the development of the immune system [72,73]. An increasing body of evidence have documented an intertwined and symbiotic relationship between host immunity and the gut microbial communities, in which beneficial and pathogenic bacteria coexist in a delicate balance. Changes in the structure of the intestinal microbiota and loss of beneficial bacterial strains shift the balance between microbiota-derived immunotolerogenic signals and microbial triggers of inflammatory responses, thereby increasing the susceptibility to or exacerbating existing inflammatory disease conditions [74–76]. Indeed, dysbiosis (microbial imbalance and maladaptation) has been associated with several immune-metabolic disorders (such as inflammatory bowel disease, obesity, diabetes, and other autoimmune diseases) and is generally defined by a drop in microbiome diversity and richness of different taxa [74–76].

Analyses of germ-free mice have helped to understand the impact of the microbiome on host physiology, especially on host immunity development. Germ-free mice exhibit immature gut-associated lymphoid tissues (GALT), fewer intestinal lymphocytes with imbalance in effector T helper cell populations, reduced immunoglobulin A production, suppression of the myeloid progenitor differentiation, alterations in expression of antimicrobial proteins and in the morphology of intestinal epithelium [77–82]. These differences between germ-free and conventional mice are mostly restored by colonization of the gut with commensal bacteria [79,80,82]. Altogether, these findings indicate that the gut microbiome directs the development of both innate and immune cells and shows that the host immune system is continuously regulated by indigenous microbes. In this regard, several studies have shown that the gut microbiota plays an important role in orchestrating T-lymphocyte differentiation [80,83–87]. For instance, studies by Ivanon et al. and Yang et al. demonstrated that single colonization with the commensal segmented filamentous bacterium drives Th17 cell differentiation in the intestinal lamina propria [83,84]. Instead, Bacteroides fragilis have been shown to promote immunosuppressive effects by augmenting the number and activity of immunotolerogenic Treg [80,86], through a mechanism that involves engagement of TLR2 by polysaccharide A from B.fragilis [88,89].

Microbiota-derived metabolites as regulators of immune responses

The modulation of the immune cell compartment by indigenous microbes occurs both at the level of the intestinal mucosa and systemically. The systemic effect on host immune physiology are thought to be largely mediated by the wide-range of microbially-derived metabolites, which after absorption can reach extra-intestinal organs [90]. In this regard, the microbial metabolism of dietary proteins and complex carbohydrates can modulate CKD/DKD progression through the production of proteolytic and saccharolytic end-products, which in turn may exacerbate or ameliorate kidney injury and dysfunction, respectively.

Short-chain fatty acids (SCFA)

SCFA, predominantly butyrate, acetate and propionate, are produced by the colonic saccharolytic fermentation of non-digestible complex carbohydrates derived by consumption of fiber–rich food and the major producers of SCFA belong to the phyla of Bacteroidetes. SCFA are used as energy source by the indigenous microbes and mainly by the intestinal epithelial cells through diffusion or co-transport [91].

SCFA have been subject of intensive research over the past years owing to their role in pacifying proinflammatory responses and promoting metabolic benefits, such as augmenting insulin sensitivity and energy expenditure, and alleviating obesity [92–94]. Mechanistically, SCFA act as histone deacetylase inhibitors (HDAC) or as ligands for G-protein coupled receptors (GPCR 43/41/109a) and the olfactory receptor Olfr78. Particularly butyrate has been shown to restrain the NFκB-elicited production of proinflammatory cytokines in myeloid cells, including macrophages, dendritic cells and neutrophils,
and promote the expansion and immunosuppressive activities of regulatory T cells \[87, 92, 95–101\]. Furthermore, butyrate plays an important role in fortifying the intestinal barrier through endorsement of mucus production and expression of tight-junction proteins by intestinal epithelial cells, and thereby preventing the leakage of microbial components into the circulation \[92\].

In the context of renal diseases, CKD patients consume less fiber due to dietary restrictions and harbor a microbiota in which SCFA-producers are constrained at the expenses of an outgrowth of microbes metabolizing dietary protein \[102, 109, 110\]. Furthermore, animal studies have demonstrated the beneficial properties of SCFA in diseased kidneys. For instance, in a murine model of acute kidney injury, Andrade-Oliveira et al. revealed that treatment with SCFA, particularly acetate, ameliorates renal dysfunction. Additionally, oral administration of the acetate-producing bacteria *Bifidobacterium longum* or *B. adolescentis*, in the days prior to kidney injury, improved kidney dysfunction and systemic inflammation \[103\]. Consistently, a high-dietary fiber intake was proven protective against renal inflammation and fibrosis also in a rat model of adenine-induced CKD \[104\]. The beneficial effects of SCFA on renal inflammation and function and their potential utilization as therapy against CKD/DKD will be later discussed in this review.

**Protein metabolism end-products**

Particularly in CKD patients, the microbial metabolism shifts from carbohydrate metabolism to protein metabolism, resulting in increased plasma levels of protein fermentation end-products, such as p-cresyl sulphate (PCS), indoxyl sulfate (IS) and trimethylamine N-oxide (TMAO). PCS, IS and TMAO are prototypical uremic toxins originating from the microbially produced precursors p-cresol, indole and trimethylamine (TMA), respectively. After absorption into the portal circulation, these precursors are converted by hepatic enzymes into the pathogenic uremic toxins. P-cresol is a microbial metabolite derived from tyrosine and phenylalanine, while indole is produced by the bacterial metabolism of the essential amino acid tryptophan. Instead, TMA is a proteolytic end-product of microbial cho line and carnitine metabolism \[105–107\]. These end-products of microbial protein fermentation are physiologically eliminated by glomerular filtration and tubular secretion. Interestingly, in a recent study, Jansen et al. revealed that kidneys possess a sensing and signaling mechanism to balance the elevation of microbial tryptophan metabolites. Jansen and colleagues also show that proximal tubular cells sense increased IS plasma levels and respond by upregulating the organic anion transporter-1 to induce IS secretion \[108\]. However, a substantial body of evidence has disclosed that upon renal function decline, these microbial metabolites build up to toxic levels in the circulation \[111–113\]. As we will later describe in this review, uremic toxins elicit an active pathogenic role in instigating glomerular and tubular damage, promoting inflammation, oxidative stress and fibrosis \[23\] (Fig. 2).

**Dysbiosis in CKD**

Previous investigations have disclosed that the intestinal microbiota of CKD patients exists in a status of dysbiosis, generally characterized by reduced richness and diversity of gut bacterial communities, along with altered microbiota composition and function.

In their pioneer study, Hilda et al. discovered an increase in enterobacteria, enterococci and bifidobacteria species and a decrease in numbers of *Clostridium perfringens* in haemodialysis (HD) patients compared to healthy controls \[107\]. In a later Chinese study, the microbiome of patients on peritoneal dialysis were reported to harbor significantly less *Bifidobacterium* species and *Lactobacillus* species, regarded as beneficial species owing to SCFA production and anti-pathogen activities \[114\]. In support of such a protective effect of *Lactobacillus* species on kidney well-being, a 2016 study showed that in hypertensive rats subjected to 5/6 nephrectomy, as model of CKD, the colonic abundance of *Lactobacillus* species was reduced compared to sham-operated rats. Moreover, therapy with supplementation of *Lactobacillus* to nephrectomized rats improved renal damage and function, alleviated systemic inflammation, assessed by serum concentrations of lipopolysaccharide, IL-6 and C-reactive protein (CRP), and also promoted the expression of intestinal tight junction components, hinting to an improved intestinal barrier integrity \[115\].

Accordingly, Vaziri and colleagues demonstrated a substantial shift in microbiota composition in advanced CKD, as they observed marked differences in the abundance of 190 bacterial operational
taxonomic units (OTUs) between the ESKD and control groups [102]. Importantly for our understanding of the gut-kidney axis, Vaziri's groups subsequently disclosed that ESKD patients exhibited a contraction of bacterial families harboring butyrate-producing enzymes and a concomitant expansion of bacterial families possessing urease, uricase, and indole and p-cresol forming enzymes, thus linking microbial dysbiosis and function to uraemia [110]. Accordingly, the abundance of two typical butyrate-producing bacteria species, *Roseburia* spp. and *Faecalibacterium prausnitzii*, were found to be diminished in the faeces of CKD Chinese patients, especially in advanced CKD stages. Moreover, *Roseburia* spp. and *F. prausnitzii* levels negatively correlated with renal dysfunction and micro-inflammation, assessed by eGFR and plasma CRP and Cystatin C concentrations [109]. Although these studies analyzed relatively small populations, altogether they attest that CKD is associated with an altered microbiome composition.

**Dysbiosis in DKD**

Accumulating evidence from the past decade has revealed that altered microbiomes are associated with metabolic disorders, such as obesity and diabetes [76]. Indeed, human studies have shown that metabolic health correlates with richness and high-diversity of the gut microbiome [18,117,118]. The landmark studies of Qin et al. and of Karisson et al. provided the first evidence that T2D subjects display intestinal dysbiosis with a reduction in prototypical butyrate-producing strains (*Clostridiales* species, *Roseburia intestinals* and *F. prausnitzii*) and enrichment in opportunistic bacteria in, respectively, a Chinese and a Scandinavian cohort [119,120]. In a very recent study harboring almost 1500 subjects, Wu et al. demonstrate that gut dysbiosis and decline in abundance of butyrate-producers occurs at both prediabetic and diabetic stages independently of glucose-lowering drugs. Remarkably, the study...
reveals that, metagenomics and functional perturbations of the microbiome species shift in parallel with impaired glycemic status [121]. Moreover, metagenomics profiles have been used to classify T2D [120,121], suggesting that microbiome signatures could be used to monitor disease state. Likewise, T1D has also been associated with intestinal dysbiosis, which is reported to anticipate disease onset [122]. These findings together with evidence from animal studies suggest that microbial maladaptations underpins, at least in part, diabetes development.

Similarly to the diabetic and CKD-associated microbiomes, a recent study revealed a reduction in gut microbiota richness in patients with T2D and biopsy-proven DKD when compared to age- and gender-matched healthy subjects. This was accompanied with variation in microbiome composition with Prevotellaceae being most abundant in healthy individuals, Actinobacteria and Bifidobacteriaceae in diabetes mellitus patients and Coriobacteriaceae differentially enriched in subjects with DKD. Importantly, renal clinical parameters were found to associate with the abundance of gut bacteria at the phylum level. Indeed, Fusobacteria negatively associated with levels of fasting blood glucose; Firmicutes negatively associated with levels of fasting blood glucose, glycosylated hemoglobin and urinary ACR; instead Verrucomicrobiota significantly correlated with eGFR. Notably, seeking for a microbial signature of the 3 groups (T2D, DKD, healthy), the authors identified that the levels of the specific genus g_Prevotella_9 accurately predicted diabetic individuals, while patients with biopsy-proven DKD compared to T2D patients without renal complications could be accurately distinguished by the variables of g_Escherichia-Shigella and g_Prevotella_9 with Escherichia-Shigella being significantly increased and Prevotella_9 significantly decreased in the DKD group [123]. To eliminate the impact of environmental confounders, such as diet, the investigators included an extra control group of households contacts and disclosed that g_Prevotella_9 richness could classify individuals with diabetes [123]. The enrichment in Enterobacteriaceae in DKD patients, when compared to healthy controls, was reported also in a smaller study by Gradisteanu et al. [124] and, as the authors postulated, the increase in the relative abundance of Enterobacteriaceae may provide a link to inflammation as they express potent immunostimulants, such as lipopolysaccharides [125].

Similarly to humans, DKD-associated dysbiosis was also documented in rodent models of diabetes with reduced alpha-diversity, a relative increase at phylum level of Firmicutes and Actinobacteria at the expenses of a relative loss of SCFA producers Ruminococcus, Rikenella and Bacteroides (B.acidifaciens species) [126]. Although human diseases cannot be exactly reproduced in animal models, these findings enforce the validity of experimental models to study the gut-kidney axis in DKD.

The gut–metabolites–kidney axis: uremic toxins

It is increasingly acknowledged that colonic microbiota contribute to CKD progression by being a major source of uremic toxins. As described previously in this review, PCS, IS and TMAO are prototypical microbiota-derived uremic toxins, which have been largely studied owing to their renal and vascular toxicity. Serum concentrations of PCS, IS and TMAO increase with the advancement of CKD [105–107,110]. In line, their concentrations rapidly decline after successful renal transplantation [111–113]. Importantly, due to their high-binding to albumin, PCS and IS are not properly cleared by conventional hemodialysis resulting in higher concentrations of these solutes in ESKD patients and a potential exacerbation of their toxic effects [127]. Instead, TMAO is completely excreted through glomerular filtration with a negligible involvement of tubular secretion and is effectively removed from the circulation by HD [128].

In support of an obligatory role for gut microbiota in the production of uremic toxins, animal studies employing germ-free mice or antibiotic treatment have proven that upon microbiota-deficiency TMAO, IS and PCS are not detectable in plasma [129]. Likewise, hemodialysis patients following colectomy display an almost absolute absence of IS and PCS [105]. Moreover, the reports of Jooddens et al. and of Gryp et al. identified the bacteria present in the fecal microbiota of CKD patients responsible for the generation of uremic toxins. The first study found that six taxa (Enterococcus, Akkermansia, Diaeter, Ruminococcus, Bacteroides and Blautia) correlated with enhanced uremic toxin concentrations [130]. In the second study instead, the investigators isolated and cultured bacteria from fecal samples of CKD patients and identified 92, out of 148 different isolated species, that produced protein-bound uremic toxins [106].
The rise in microbial uremic metabolites is typically explained by decline in renal metabolite clearance and concomitant increase in colonic production, which is caused by a shift from saccharolytic to proteolytic fermentation in the CKD-microbiota. This phenomenon is thought to result from the chronic uremic status, dietary limitations and altered intestinal transit time in CKD [102,105,110]. Indeed, the influx of urea and uremic toxins into the GI lumen may apply a selective pressure to the microbiome community leading to the outgrowth of bacteria possessing urease and uricase [102,110,113]. In line, patients with DKD present increased levels of urea in the circulation as well as in the intestinal lumen [131]. Concomitantly, the nutritional management of CKD patients, characterized by a drastic fiber restriction to control potassium levels, favors a microbial imbalance toward more proteolytic microbiota and less saccharolytic microbes, with thus less SCFA production [109,113,132–135]. Lastly, the prolonged colonic transit time observed in CKD patients promotes an increase in protein and carbohydrate fermentation in the small bowel, hence reducing the availability of carbohydrates to colonic saccharolytic bacteria [133,135–137].

**Microbial uremic metabolites in CKD and DKD**

Several studies have reported an association between circulating rates of uremic toxins and increased risk of mortality, cardiovascular events and progression of renal disease in CKD patients [138]. Herein, we will describe the findings of some of these studies in relation to CKD (Table 1).

**Table 1**
Overview of relevant metabolites and bacteria.

| Metabolites        | Characteristics                                      | Functional effects and/or relevance                              | CKD                                      |
|--------------------|------------------------------------------------------|----------------------------------------------------------------|------------------------------------------|
| p-cresol           | Bacterial fermentation metabolite of tyrosine        | Induction of inflammatory reactions and renal and cardiovascular impairment | [209,210]                                |
| Indoxyl-sulphate   | Metabolite of dietary L-tryptophan                   |                                                                  |                                         |
| TMAO               | Gut microbe-dependent metabolite, produced from degradation of choline and L-carnitine | Associated with higher risk of progressive renal fibrosis and functional impairment | [153,160]                                |
| SCFA               | Fiber fermentation metabolite                        | Protection against CKD trans-membrane G protein-coupled receptor activation | [212,213]                                |
| Organism           |                                                      |                                                                  |                                         |
| g_Prevotella_9     | Gram-negative bacteria, mainly present as oral, vaginal, and gut microbiota | Distinguish patients with biopsy-proven DKD compared to T2DM patients without renal complications | [116,123,214]                            |
| g_Escherichia-Shigella | Gram-negative pathogen in humans                       |                                                                  |                                         |
| Faecalibacterium prausnitzii | Gram-positive bacteria, one of the most abundant and important commensal bacteria of the human gut microbiota | Known as human gut colonizers and butyrate producers, strictly differentiating for T2D | [109,214] |

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In a subset of 394 HD patients from a multicenter prospective cohort (CHOICE study), the combined solute index of 4 free-plasma uremic solutes (PCS, IS, hippurate and phenylacetylglutamine) was found to be associated with higher creatinine and urea levels as markers of renal failure. In addition, patients with the highest concentrations of all 4 metabolites had a 96% greater risk of cardiovascular mortality, whereas a high PCS rate alone correlated with increased risk of cardiovascular mortality and first atherosclerotic cardiovascular event [139]. In a separate study employing 521 incident hemodialysis patients from the CHOICE cohort, elevated IS levels were associated with all-cause mortality, but not with cardiovascular mortality [140]. These findings are in line with the meta-analysis conducted by Lin et al. Using data from 11 distinct studies and a total of 1572 patients at different stages of CKD, the authors report that the rise in PSC and IS are significantly associated with elevated mortality among CKD patients [141].

Importantly, the toxicity of uremic metabolites seems accounted by their free forms. In fact, Sahfi et al. could not confirm the association of total (free and protein-bound) plasma PCS and IS with risk of cardiac death in 1273 HD patients from the multicenter HEMO study; nonetheless the association became significant when studying a sub-group of patients with low-albumin levels [142]. Thus, albumin-free uremic metabolites may be better indicators of adverse clinical outcome. In a very recent study by Wang et al., multidimensional data integration of the microbiome profiles, serum and fecal metabolomes in 223 ESKD patients and 69 healthy controls revealed a tight link between fecal and serum metabolite landscapes, which were in turn associated and determined by the gut microbiota profiles. Indeed, in ESKD patients, the fecal and serum metabolomes were found enriched in uremic toxins and secondary bile acids, in association with an ESKD-associated enrichment of specific intestinal microbes, such as *Eggerthella lenta* and *Fusobacterium nucleatum*. The authors further link the aberrant microbiome composition with the renal disease state, as intestinal engraftment with the 2

| Table 1 (continued) |
|---------------------|
| Metabolites | Characteristics | Functional effects and/or relevance | CKD |
| **Roseburia spp** | Gram-positive anaerobic bacteria, inhabit the human colon |  | [109,116,214] |
| **Bacteroides** | Gram-negative bacteria, symbiotic host—bacterial relationship with humans | Predominant organisms, capable to develop and multiply in the gastrointestinal tract as a consequence of delayed intestinal transit time of contents as a result of intestinal motility disorder | [214,215] |
| **Fusobacterium** | Gram-negative bacteria, pathogenic strains can cause several human diseases |  | [214,216] |
| **Eubacterium** | Gram-positive bacteria in the family Eubacteriaceae |  | [214,217] |
| **Bifidobacterium** | Gram-positive bacteria, kolonize the gastrointestinal tract microbiota in mammals |  | [214,218] |
| **Prevotella copri** | Gram-negative, common intestinal bacteria | Role in insulin resistance improvement | [143,219,220] |

Abbreviations: TMAO = Trimethylamine-N-oxide, SCFA = Short Chain Fatty Acid.
species or with whole feces from ESKD subjects increased uremic toxins production and prompted renal disease in murine models of CKD [143].

In the context of DKD, PS has been shown to be a microbial marker of DKD progression. With both observational and functional data, the study of Kikuchi et al. provides evidence of the role of the gut–metabolite–kidney axis in DKD progression. In a cohort of 363 diabetic patients, Kikuchi et al. found that the serum concentration of the uremic toxin phenyl sulfate (PS) correlates with urinary ACR and moreover predicts the 2 year ACR deterioration, especially in DKD patients with microalbuminuria. Utilizing murine models of mild (db/db and HFD-fed KKAy mice) and severe (eNOS-KO/Akita mice) DKD, Kikuchi et al. also demonstrate an active pathogenic role of PS in DKD, as PS administration aggravated albuminuria by eliciting podocyte damage and proinflammatory and profibrotic effects. Furthermore, they showed that PS is a modifiable therapeutic target. Indeed, inhibition of the microbiota-specific enzyme tyrosine phenol-lyase, responsible for the synthesis of the PS precursor phenol, provided renoprotection in db/db mice as shown by a reduction of albuminuria, plasma creatinine, and rates of uremic toxins PS and IS [144].

Multiple studies have indicated that uremic toxins elicit deleterious effects in several cell-types [145]. IS and p-cresol have been shown to inhibit endothelial cells proliferation, impair endothelial wound repair and enhance ROS production [146–148]. Of importance for DKD, the study of Yu et al. further suggest that IS is accountable for renal endothelium dysfunction, as the authors show that IS levels correlate with diminished rates of flow-mediated endothelium-dependent vasodilatation in 40 CKD patients and, in vitro, IS inhibited endothelial cell proliferation and production of nitric oxide while inducing oxidative stress and cellular senescence in endothelial cells [147]. Similarly, investigations on the effect of uremic toxins on renal proximal tubular cells have revealed that PCs and IS trigger a proinflammatory and pro-fibrotic response in tubular cells and also provoke oxidative stress by lowering the antioxidant response [149–152].

Similarly to PCs and IS, TMAO rates predict poorer long-term survival in CKD patients [153–155]. In a systemic review and dose-dependent meta-analysis of 17 clinical studies (including CKD and non-CKD populations), Schiattarella et al. reported that the relative risk for all-cause-mortality rises by 7.6% per each 10 μM increment in TMAO [154]. In line, another meta-analysis of 19 prospective studies by Heianza et al. has further underscored the association between elevated blood concentrations of TMAO and TMA with major cardiovascular event and mortality [155].

The clinical study of Tang et al., comprising 3687 subjects, revealed that plasma TMAO concentrations are correlated with declined eGFR and higher plasma levels of cystatin C and high TMAO is predictive of all-cause mortality at 5 years follow-up [153]. In the same report, Tang et al. provide insight into the causality between TMAO and progressive kidney diseases, as the authors show that feeding mice with TMAO or its precursor choline augmented serum rates of the inflammatory marker cystatin C and strikingly induced significant tubular injury and renal fibrosis, indicating a direct contribution of TMAO to the progression of renal disease [153]. In line, Sun et al. discovered that obese mice display substantially higher plasma TMAO than lean mice, and that treatment with the TMA formation inhibitor 3,3-Dimethyl-1-butanol reduced TMAO levels and prevented HFD-induced renal alterations, including renal fibrosis, expression of the kidney injury molecule-1 (typically used as renal damage biomarker) and of pro-inflammatory cytokines [156]. The study of Xu et al. confirmed the marked rise in plasma TMAO in CKD patients with a median concentration of 30 μM in CKD patients and 2 μM in healthy controls and further that elevation in plasma TMAO was transferable to conventional mice by transplantation of fecal samples from CKD patients [113]. Overall these studies indicate a prognostic value of high TMAO levels for poorer long-term survival and a pathogenic role for TMAO in advancing CKD. Notably, elevated systemic TMAO rates have been associated with T2D and altered glucose metabolism and this relation is more pronounced in subjects with modestly impaired renal function [157–160]. However, functional studies directly linking TMAO to adverse outcome in DKD are yet lacking.

Finally, these clinical and experimental findings highlight how an aberrant microbiota composition and function sculpt a pathogenic metabolome aggravating renal disease progression.
The gut—metabolites—kidney axis: SCFA

SCFA & kidney disease

As described above, the colonic microbiota is particularly important in the production of the beneficial SCFA from the metabolism of fiber-rich food. Consistent with the previously reported protective actions of SCFA against inflammatory and metabolic diseases, Andrade-Oliveira et al. showed that SCFA treatment is effective in ameliorating renal dysfunction in a murine model of ischemia-reperfusion injury, and in dampening systemic and renal proinflammatory cytokine production [103]. Accordingly, in a rat model of adenine-induced CKD, high amylose resistant starch diet (rich in fiber) alleviated oxidative stress, inflammation and fibrosis in kidneys [104]. More importantly, a large retrospective study evidenced that total fiber intake is particularly low in CKD patients compared to the general population and inversely related with CRP levels and mortality among individuals suffering from kidney disease [161]. This is in line with the reports published by Vaziri’s group in which a constriction of SCFA-producing bacteria was documented in a ESKD rat model as well as in patients suffering from ESKD [102,110]. Accordingly, Noureldein et al. determine that the taxonomic composition of microbiota changes with disease severity and CKD patients mainly present lower levels of butyrate-producing bacteria and higher levels of potential pathogens were detected in CKD patients [162]. Furthermore, a case control study to human gut microbiota in 65 CKD patients showed a negative correlation between butyrate-producing bacteria and higher levels of potential pathogens were detected in CKD patients [162].

SCFA protective functions in experimental DKD

The recent study by Li et al. provides important insight into the gut-kidney interrelationship and most importantly into the protective functions of renal SCFA signaling against DKD [126]. Using a murine model of streptozotocin-induced diabetes, the authors demonstrate that dietary manipulation of the gut microbiome with 2 types of fiber-rich diets (enriched in resistant starch or guar gum and cellulose) protect against the clinical and histological manifestations of DKD, including albuminuria, glomerular hypertrophy, mesangial expansion, podocyte loss, tubulointerstitial fibrosis, macrophage recruitment and production of pathogenic proinflammatory cytokines, despite equivalent hyperglycemia degrees as the mice groups fed a normal chow diet or zero-fiber diet. Notably, the high-fiber diets improved the diabetes-associated dysbiosis reducing the growth of the urease-positive pathobiont Bilophila wadsworthia and supporting the expansion of SCFA-producing genera Prevotella and Bifidobacterium with a robust increase in fecal SCFA levels. Additionally, the authors provide direct evidence that the SCFA butyrate and acetate are key mediators of these beneficial effects through signaling via their receptor GPR43 and GPR109A. In fact, SCFA supplementation reproduced the amelioration of DKD observed in high-fiber diet-fed diabetic mice, whereas the benefits of acetate were abrogated in Gpr43−/− mice and butyrate induced only a partial protection of kidney function in absence of GPR109A [126].

In line, the findings of Felizardo and co-authors prove a protective effect of bacterial butyrate against proteinuria, glomerulosclerosis, renal fibrosis and inflammation in a model of Adiriamycin-induced proteinuric kidney disease. Furthermore, the authors disclosed that butyrate hampers nephropathy progression via activation of GPR109A, in accordance to the study of Li et al., as well as via epigenetic modifications [163]. Nevertheless, the pathways triggering epigenetic modifications in response to microbial metabolites deserve further investigations in the context of DKD, especially in light of studies disclosing perturbations of HDAC activities in diabetic kidneys and the therapeutic benefits of various HDAC inhibitors in experimental DKD models [164].

Importantly, butyrate has been proven to play a key role in the cellular defenses against oxidative stress, which largely account for renal damage in DKD. In this regard, butyrate is known to activate the transcription factor NRF2 (nuclear factor erythroid 2—related factor 2), an intracellular governor of antioxidant signaling [165–167]. Using a streptozotocin-induced diabetes model in wild-type and Nrf2-knockout mice, Dong et al. demonstrated that butyrate, supplemented in standard diet, prevented the expansion of glomerular and mesangial area in DKD, interstitial fibrosis, oxidative damage in a NRF2-dependent manner [168]. Similarly, Huang and colleagues found that the SCFA acetate and
butyrate or GPR43 agonist can inhibit the proliferations of glomerular mesangial cells and diminish their oxidative stress level caused by high-glucose or LPS exposure [169].

Altogether these studies highlight the importance of maintaining the delicate balance among intestinal microbial communities and demonstrate that fiber-rich diets or butyrate/acetate supplementation may serve as effective treatments against glomerulopathies and proteinuric kidney diseases.

**Dysbiosis-driven inflammation in CKD and DKD**

Increasing evidence indicate that gut dysbiosis plays a key role in the pathogenesis of chronic systemic inflammation. An overrepresentation of gram-negative bacteria in the microbial populations in diabetic CKD patients is accompanied by a significant elevation in the serum levels of the potent immunostimulant LPS, present in the outer membrane of Gram-negative bacteria, which correlates with inflammatory markers, such as tumor necrosis factor-α (TNFα), IL-6 and CRP [170]. Similarly, the outgrowth of bacteria with proteolytic activities that promote the generation of the uremic toxins IS, PCS, indole-3-acetic acid (IAA), and TMAO is also accountable for instigating inflammatory and oxidative stress responses [171]. Finally, the expansion of bacteria possessing urease enzymes in CKD patients results in increased ammonia concentrations and pH in the gut lumen, consequently leading to mucosal irritation and enhanced intestinal permeability [110,172] (Fig. 2).

The impairment of the intestinal barrier function in CKD is not a new concept, as early studies from the 80s and 90s have reported alterations of the intestinal barrier in CKD patients [173,174]. Later studies showed that serum levels of bacterial and fungal components and of the endotoxin ligand soluble CD14 are elevated in CKD patients [175–179]. In addition, using two rat models of CKD (5/6 nephrectomy and adenine-induced CKD), Vaziri et al. showed that, upon uremia, the intestinal barrier is disrupted by marked depletion of tight-junction proteins and accumulation of leukocytes in the colonic lamina propria [180]. In line, Wang et al. detected in the blood of a portion of nondialyzed CKD patients (6 out of 30) bacterial DNA that matched the bacterial genera (Klebsiella spp, Proteus spp, Escherichia spp, Enterobacter spp, and Pseudomonas spp) overgrown in the guts of these ESKD patients, suggesting bacterial translocation. Indeed, plasma D-lactate, used as marker of gut permeability, CRP and IL-6 levels were significantly higher in CKD patients with bacterial DNA in the circulation as compared to the control group and ESKD without blood bacteria DNA [181]. We can assume that, once the intestinal barrier is compromised, the release of microbial immunogens, such as LPS and peptidoglycans, can trigger, upon TLR-engagement, pro-inflammatory cascades in kidneys and hence aggravate CKD [175,176]. Overall, this state of dysbiosis and chronic inflammation constitutes a risk factor for CKD progression and vascular complications [178,182,183]. Importantly, the “leaky gut” phenomenon is also typical in obese and diabetic individuals, stressing the importance of this pathways in DKD [76].

Innate PRR receptors are deeply involved in kidney injury. This is particularly relevant in the context of DKD as expression of TLR2 and TLR4 is upregulated in renal cells upon hyperglycemia and their engagement drives diabetes-induced renal inflammation. Indeed, animal model of STZ-induced DKD using TLR2/4-deficient mice unraveled the implication of TLR-signaling not solely in the development of renal inflammation but most importantly glomerular and tubular injury, fibrosis and lastly in the onset of albuminuria [55,62,184]. Thus far, the deleterious effects of TLR activation in diabesity-induced nephropathy have been largely attributed to the accumulation of endogenous nonmicrobial agonists, such as HMGB1, biglycan and Hsp70 [54,55,184]. Nonetheless, in light of mounting evidence signifying loss of intestinal barrier integrity in CKD (as described above), the augment of microbiome-derived products in the circulation is likely to results in the engagement and activation of renal TLRs and thereby contribute to renal inflammation and injury in diabetic kidneys.

The proinflammatory effects of TLR signaling in kidney diseases may be counteract by SCFA sensing by renal cells. In fact, in murine DKD, besides reducing the macrophage accumulation within diabetic kidneys, high-fiber diet repressed the production of pathogenic proinflammatory cytokines and the expression of the innate immune receptors TLR2 and TLR4 within diabetic kidneys [126]. Accordingly, intraperitoneal injection of the SCFA acetate inhibited the expression of Tlr4 and of its endogenous ligand biglycan in ischemic kidneys [103]. Remarkably, in this study, the intraperitoneal administration
of acetate hampered not solely the recruitment of myeloid cells but also the degree of activation of macrophages and dendritic cells upon acute kidney injury. Furthermore, in vitro analyses revealed that SCFA butyrate, acetate and propionate impaired the maturation state of dendritic cells and their antigen-presenting abilities as SCFA-treated dendritic cells displayed lower capacity of inducing CD4 and CD8 T cell proliferation [103]. As enhanced trafficking and activation of T lymphocytes exacerbate renal dysfunction in DKD, preventing the activation of dendritic cells in the renal environment represents a critical aspect in the multifaceted protective role of SCFA against renal function deterioration. In line, Huang and coauthors reported that, upon hyperglycemia or LPS, high-glucose or LPS, treatment with acetate, butyrate or GPR43 agonist inhibit inflammation in glomerular mesangial cells by suppressing the expression of intercellular adhesion molecule-1 (ICAM-1), monocyte chemoattractant protein-1 (MCP1) and IL-1β [169]. Altogether, these findings indicate that SCFA sensing by renal cells diminishes their ability to recruit leukocytes into kidneys harnessing the expression of adhesion and chemoattractant molecules. Furthermore, SCFA may suppress renal activity of the innate NLRP3-ASC-pro-caspase-1 inflammasome complex. This is of particular importance since NLRP3, expressed by resident glomerular and tubular cells, can be activated by AGEs and mitochondrial ROS during hyperglycemic conditions [68,185]. Furthermore, the elegant study of Shahzad et al. revealed that the activation of the ROS-NLRP3 signaling within glomerular resident endothelial cells and podocytes plays a fundamental role in the pathogenesis of diabetic nephropathy [68].

The ROS-NLRP3 pathway can be counteracted by SCFA, indeed antioxidant effects of SCFA have been documented in murine proteinuric kidney disease [103,163,169]. In support of the inhibitory action of SCFA on the inflammasome cascade, butyrate was reported to suppress NLRP3 inflammasome activation via antioxidant activities in an in vivo model of acute endothelial dysfunction and in response to atherogenic stimuli [186]. However, so far it remains to be elucidated whether SCFA directly regulate PRR expression and signaling in diabetic kidneys.

A plausible connection between gut microbiota and NLRP3-mediated injury in DKD may be given by elevated TMAO levels. In fact, numerous studies have shown that TMAO can trigger oxidative stress and, hence, NLRP3 inflammasome activation and secretion of IL-1β and IL-18 in endothelial cells [187–190]. Thus, upon hyperglycemic conditions, TMAO may elicit pro-inflammatory effects in glomerular endothelial cells in part through NLRP3 activation.

Accordingly, Chen et al. disclosed that inhibition of TMAO formation by oral administration of % 3,3-Dimethyl-1-butanol prevents Western diet-induced increase in plasma TMAO and expression of proinflammatory TNF-α and IL-1β and interstitial fibrosis in the heart [191]. Hence, a similar approach may be beneficial in restraining NLRP3-driven damage in DKD. Similarly, the uremic metabolites IS, PSC have been shown to trigger oxidative stress and proinflammatory responses in immune, endothelial and tubular cells; hence, they may also contribute to NLRP3 inflammasome activation [145,192–195].

Overall, whereas SCFA hamper proinflammatory signaling, the microbiobially produced uremic toxins promote the secretion of cytokines and chemokines, such as MCP-1, and the upregulation of leukocyte adhesion molecules (E-selectin, ICAM) by renal parenchymal cells, thereby facilitating leukocyte recruitment and deteriorating kidney dysfunction [151,156,192,196]. Thus, interventions aiming at reducing the levels of microbial uremic toxins represent a promising therapy in both CKD and DKD. Interestingly, in the innovative study of Devlin et al., IS production was harnessed via colonization of germ-free mice with a Bacteroides species, B. theta, harboring a deletion of the tryptophanase gene BT1492, or diet supplementation with fructooligosaccharides, which favors the growth of nonindole producers, such as B.caccae [197]. Although far from an actual clinical intervention, this study may pave the way to future therapeutic approaches specifically targeting groups of microbes concordant in metabolic properties.

Future perspectives: gut microbes as therapeutic targets?

Interventions targeting the microbiota as a whole or specifically modulating some of its function constitute an attractive approach against CKD and DKD progression. As diet is a major determinant of microbiota composition, changes in dietary habits (e.g. higher fiber intake and lower protein intake) represent a key manner to reshape the gut microbiota. As discussed in this review, several studies have shown the protective effects of dietary fibers and of SCFA against DKD.
Although clinical evidence on the effects of diet on kidney function in DKD patients is limited, data from clinical trials indicate that fiber supplementation decreases blood urea and creatinine levels and improves eGFR in CKD patients [198,199]. Moreover, the protein-fiber intake ratio was reported to associate with systemic levels of uremic toxins in CKD, indicating the importance of profound shifts in dietary regimens [200]. Undoubtedly, the SCFA-induced signaling in the injured kidney warrants further investigations and the development of strategies to boost SCFA production (e.g., fiber supplementation, engineering of bacteria to express key enzymes for SCFA production) may greatly advance current therapy to counteract and/or prevent DKD.

Prebiotics, probiotics and synbiotics have been tested in the past to promote a healthy microbiome and reduce the levels of uremic toxins. The use of prebiotics, such as arabic gum and oligofructose, in CKD patients harbored some positive clinical effects, namely a rise in the abundance of saccharolytic bacteria and of fecal nitrogen excretion and a reduction in the uremic solute PCS [201,202].

Regarding the clinical use of probiotics, some controversies arise from the literature. In the study of Hilda et al., oral administration of lactic acid bacteria was found to suppress the growth of pathogenic aerobes enterobacteria (enriched in HD patients) together with the fecal concentrations of the microbial metabolites p-cresol and indole in HD patients after 2 weeks of probiotic therapy [107]. However, in a randomized, placebo-controlled, crossover study encompassing 22 ESKD patients, 2-month therapy with a probiotic formulation (30 billion CFU of *Streptococcus thermophilus* KB 19, *Lactobacillus acidophilus* KB 27, and *B. longum* KB 31) did not result in significant variations in uremic toxin rates nor in markers of inflammation and oxidative stress [203]. Similarly, a 12-week regimen of a
probiotic mixture, containing 4 Lactobacillus strains, 3 Bifidobacterium species and one Streptococcus salivarius subsp., in ESKD pediatric patients did not diminish the levels of PCS and IS [204]. Nonetheless, some beneficial effects have been obtained by combining prebiotic and probiotic components (synbiotic therapy). For instance, the study of Rossi et al. showed that synbiotic therapy resulted in a decline of both PCS and IS concentrations and an enrichment of Bifidobacterium in the fecal microbiome of CKD patients [205]. Similarly, Nakabayashi et al. reported a decrease in PCS generation rate and serum concentration after 4-week symbiotic therapy [206].

A more durable and effective therapeutic strategy to restore a healthy microbiome structure and function may comprise sequential fecal microbiota transplantations (FMT) from healthy donors possibly in conjunction with low-protein, high-fiber dietary regimens (Fig. 3). Using an animal model of adenine-induced CKD, Bara et al. evaluated the effect of healthy FMT after induction of kidney failure. The authors found that FMT from healthy mice reversed some of the CKD-microbiota disturbances, enhanced the microbiota alpha-diversity, and, at systemic levels, FMT improved PCS accumulation, glucose tolerance and albuminuria. However, FMT, at 40 days after adenine supplementation, did not reduce serum creatinine levels [207]. In patients, the reestablishment of a “healthy microbiota” can potentially restore intestinal, immune and metabolic homeostasis and has already been proven safe and well-tolerated in previous clinical trials [21,22,208]. Moreover this type of clinical research is also a great opportunity to better understand the complicated interactions between microbiome, immunity and kidney responses and may lead to the identification of indigenous microbes with a beneficial association with kidney function or of novel microbial metabolic pathways that can be targeted in future interventions.

Altogether, more research is needed in this field; in particular, this field is in need of large human studies in which bacterial metagenomic analysis is coupled to plasma proteomics and metabolomics to dissect the impact of specific microbial signatures on the systemic metabolite and protein landscapes in DKD patients. Furthermore, it would be advantageous to investigate the pathogenic role of dysbiosis in DKD progression by transferring fecal samples from healthy subjects or DKD patients, at different stages of CKD, into mice subjected or not to experimental models of DKD. These types of experiments are necessary to prove causality between an aberrant microbiota and disease severity or disease onset.

Summary

In conclusion, in the gut-kidney crosstalk the healthy states of the intestinal microbiome and of kidneys mutually depend on each other; the own condition of kidney injury/dysfunction predisposes to the loss of protective saccharolytic bacteria in the gut, and the disequilibrium in gut commensals favors the progression of kidney disease through large production of uremic toxins.

Our understanding of the gut-kidney crosstalk is still at its infancy, although knowledge is rapidly accruing. More efforts are required to identify the pathophysiological components in this mutual relationship and to establish novel therapeutic strategies to harness the vicious cycle of CKD/DKD-gut dysbiosis that advances kidney disease to ESKD.

Practice points:

- Changes in microbiota composition and function should be considered as risk factors and therapeutical targets in the management of CKD/DKD.
- Circulating levels of gut-derived uremic toxins should be measured to help assessing the risk of CV and renal complications in diabetic patients.
- DKD is largely driven by inflammatory reactions; scientific evidence shows that targeting TLRs and inflammasomes represents promising strategies to improve clinical and pathological parameters in DKD.
Research agenda:

- To identify a structural and functional microbial signature specific to DKD-dysbiosis, we propose the future performance of multiomics cross-sectional studies enrolling healthy-controls, diabetic, CKD and DKD patients, in which bacteria metagenomics analysis is integrated with plasma metabolomics and proteomics.
- To unravel whether early variations in microbiota composition and functionality are predictive of DKD progression, similar multiomic approaches can be implemented in longitudinal studies enrolling diabetic individuals and DKD patients.
- To establish the pathogenic processes linking an aberrant microbiota to DKD severity, FMTs from either healthy donors or DKD patients should be performed in experimental murine models of DKD.
- Lastly, the findings from the suggested clinical and experimental studies should converge in the development of microbiome-targeting strategies aiming to restore a healthy microbiota and to halt the systemic inflammatory tone and microbial production of uremic toxins in DKD.

Acknowledgements

The writing of this review was funded by a Top Consortia for Knowledge and Innovation (TKI-PPP) grant (Health-Holland, 2020) awarded to E.Rampanelli.

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