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MICROBIOTA METABOLITES: PIVOTAL PLAYERS OF CARDIOVASCULAR DAMAGE IN CHRONIC KIDNEY DISEASE

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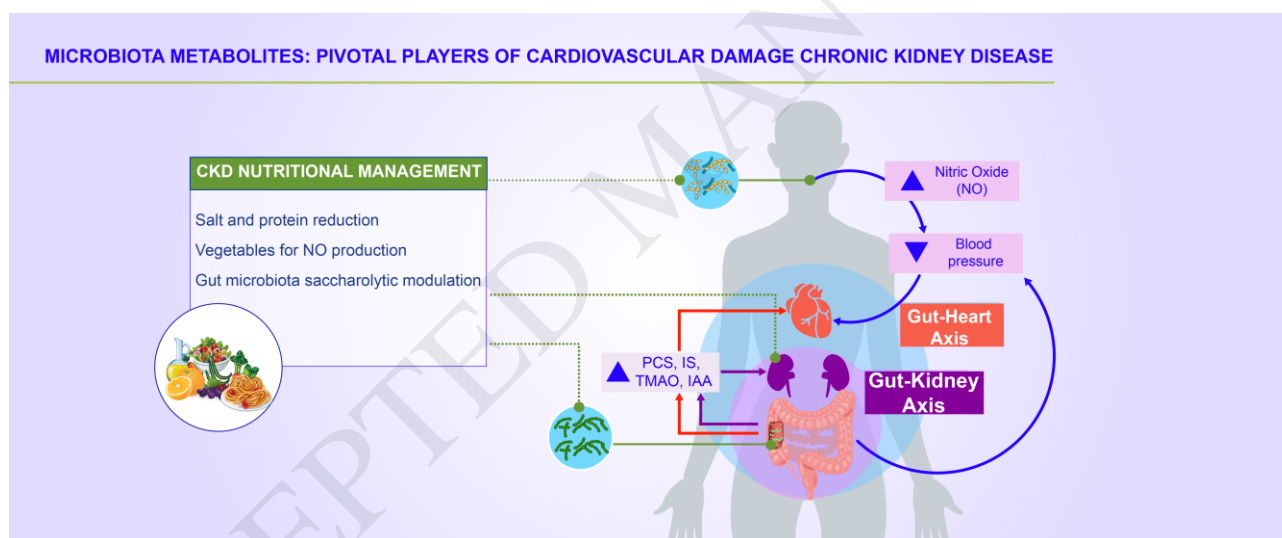
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Graphical abstract



Abstract

In chronic kidney disease (CKD), cardiovascular (CV) damage is present in parallel which leads to an increased risk of CV disease. Both traditional and non-traditional risk factors contribute to CV damage in CKD. The systemic role of the microbiota as a central player in the pathophysiology of many organs is progressively emerging in the literature: the microbiota is indeed involved in a

complex, bi-directional network between many organs, including the kidney and heart connection, although many of these relationships still need to be elucidated through in-depth mechanistic studies.

The aim of this review is to provide evidence that microbiota metabolites influence non-traditional risk factors, such as inflammation and endothelial dysfunction in CKD-associated CV damage.

Here, we report our current understanding and hypotheses on the gut-kidney and gut-heart axes and provide details on the potential mechanisms mediated by microbial metabolites. More specifically, we summarize some novel hypotheses linking the microbiota to blood pressure regulation and hypertension. We also emphasise the idea that the nutritional management of CKD should be redesigned and include the new findings from research on the intrinsic plasticity of the microbiota and its metabolites in response to food intake. The need is felt to integrate the classical salt and protein restriction approach for CKD patients with foods that enhance intestinal wellness. Finally, we discuss the new perspectives, especially the importance of taking care of the microbiota in order to prevent the risk of developing CKD and hypertension, as well as the still not tested but very promising CKD innovative treatments, such as postbiotic supplementation and bacteriotherapy.

This interesting area of research offers potential complementary approaches to the management of CKD and CV damage assuming that the causal mechanisms underlying the gut-kidney and gut-heart axes are clarified. This will pave the way to the design of new personalized therapies targeting gut microbiota.

Keywords

chronic kidney disease, microbiota, cardiovascular damage, nutritional management, uremic toxins, nitric oxide

Introduction

Cardiovascular (CV) damage is one of the main concerns in the clinical management of chronic kidney disease (CKD). Indeed, CKD patients are exposed to a 2- to 4- fold higher risk of

cardiovascular diseases (CVD) than the general population and are more likely to die from CVD than from CKD [1,2]. CVD incidence is not sufficiently accounted for and supported by the traditional risk factors [3]. Quite the contrary, many emerging non-traditional risk factors, including endothelial dysfunction and inflammation, are being recognized as co-responsible in the aetiology of CV damage [4]; in addition there is increasing evidence that the microbiota should be considered a key player in this setting, since its metabolites influence CKD progression and the aforementioned non-traditional risk factors [3-5].

Moreover, novel indications suggest that the gut and even oral microbiota may play a role in blood pressure (BP) control and in the aetiology of hypertension [6,7]. Hypertension is a frequent comorbidity and a risk factor associated with CKD, also in light of the major role of the kidney in BP regulation. The new evidence for an association between hypertension and gut microbiota dysbiosis adds a new tile to the complex frame of the gut-kidney axis.

In this review we discuss the role of the microbiota and its bioactive metabolites as a key player in the social network represented by gut-kidney-heart axis, and we summarize the current understanding and hypotheses on the role of gut microbiota as a non-traditional risk factor for CV damage. In addition a review of some literature studies on the enterosalivary NO pathway where oral microbiota is a key actor is also provided. What is more, molecular details in support of the putative mechanisms of uremic toxins-induced increase in CV risk are given together with an overview of the future perspectives in microbiota modulation, both in terms of prevention and therapy. Indeed, in light of the high plasticity of the gut microbiota in response to diet, and in consideration of the fundamental importance of nutrition in CKD, the nutritional management and the pro-, pre-, syn- and postbiotic supplementation offer an enormous potential in terms of reduction of CV damage by targeting the gut.

The microbiota issue in CKD: the gut-kidney axis

Increasing evidence is demonstrating the presence of a variety of relationships between the gut microbiota and extra-intestinal organs, including the kidney, heart and brain, often referred to as “axes” [5,7-9]. A complex, bi-directional relationship between the gut microbiota and the kidney

does exist. This is true in physiological conditions, with the microbiota influencing metabolism and immunity and the kidney dealing with microbial metabolites, but becomes crucial in pathology [5,8]. Indeed, during manifest CKD, especially in advanced stages, the kidney is not anymore able to deal with microbial-derived uremic toxins (UTs) that start to accumulate in the blood triggering a cascade of inflammatory and oxidative reactions [5] and inducing pro-fibrotic effects [10-12] likely to be responsible for renal damage progression. Several studies in CKD patients have investigated the relationship between increased serum levels of microbial-derived UTs and CKD progression [13,14], even though these associations have not yet been conclusively established [15]. On the other hand, it is the same uremic status that induces a shift in the gut microbial metabolism and composition [16], and the resulting dysbiosis is often worsened, especially in advanced CKD, by the current nutritional management which is characterized by a drastic fiber restriction that favours the proteolytic imbalance [17].

Some studies have been focused on the characterization of gut microbiota dysbiosis in CKD. Studying microbiota in haemodialysis (HD) patients, Hida *et al.* found an increase in enterobacteria and enterococci, accompanied by a decrease of bifidobacteria compared with controls [18]. In a more comprehensive study conducted on end-stage renal disease (ESRD) patients, the group of Vaziri highlighted an abundance of 190 bacterial operational taxonomic units from *Brachybacterium*, *Catenibacterium*, *Enterobacteriaceae*, *Halomonadaceae*, *Moraxellaceae*, *Nesterenkonia*, *Polyangiaceae*, *Pseudomonadaceae*, and *Thiothrix* families, compared with healthy subjects [16]. In a metabolomic characterization study by the same group, an expansion of bacterial families possessing urease, uricase, and indole and p-cresol forming enzymes, and a reduction of families possessing butyrate-forming enzymes was observed in ESRD [19]. Overall these results indicate a shift towards the proteolytic metabolism which results in increased UTs production.

The cellular toxicity of protein-bound uremic toxins

The impact of protein-bound UTs on vital processes and on clinical outcomes in CKD has long been neglected, thus delaying the development of intervention strategies suitable to reduce their plasma levels to alleviate their potential toxic effects.

Indoxyl sulfate (IS) and p-cresyl sulfate (PCS) are the prototypes of the intestinal-derived protein-bound UTs that are currently most studied in the context of gut-kidney axis and CV risk [20-24].

Due to their reduced renal clearance, they progressively accumulate in CKD patients: many biological and toxic effects have been attributed to them [20].

Actually, colonic microbes produce a large number of uremic solutes. Larger populations of CKD patients with colectomies and the use of complementary LC-MS-based analysis methodologies would be required in order to obtain a full profile of the microbial-derived compounds retained in uremia. In fact, most uremic solutes can go unidentified because they cannot be found in standard metabolomic databases, as shown in a study comparing plasma from HD patients with and without colons, which has identified 5 of 30 colon-derived uremic solutes, including IS, PCS, indoxyl glucuronide, indoleacetic acid, p-cresyl glucuronide, phenyl sulfate, phenyl glucuronide, phenylacetic acid, phenyl acetyl glutamine, hippuric acid and 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid [25]. The high affinity of some UTs with serum proteins, such as albumin, makes them difficult to eliminate with standard dialysis when their serum levels increase, as it happens in CKD. Their accumulation, beyond their intrinsic toxicity, results in organ damage. Here we summarize the molecular basis of the cellular toxicity of the protein-bound UTs IS, PCS, and of the less investigated Indole Acetic Acid (IAA), all potentially contributing to an increased CV risk in CKD. Table 1 summarizes their synthesis and excretion pathways.

IS has been measured and observed to increase in the blood of patients with CKD since the 60s [26]. IS has a molecular weight of 213 Da and is a small solute deriving from the metabolism of dietary tryptophan, which is converted into indole by the tryptophanase present only in colon resident microbes. Indole is absorbed in the colon and then enters the systemic circulation where further oxidation and sulfation reactions in the liver form IS [5]. IS is taken up by organic anion transporters (OAT1 and OAT3) [27] placed on the basolateral membrane of tubular cells to be secreted in the urine. The interest in PCS as such is relatively more recent [28], since the volatile

p-cresol started to be investigated, instead of its sulfate ester, in the blood of uremic patients in the 70s [29]. PCS, like IS, has a low molecular weight (188 Da) and originates exclusively in the colon from dietary nutrients deriving from the conversion of tyrosine and phenylalanine to p-cresol by anaerobic intestinal bacteria [5]. P-cresol is then absorbed in the gut and sulfated in the enterocytes and in the liver [30]. As IS, PCS is filtered by glomerular capillaries to the tubular cells through OAT-1 and OAT-3 to end-up in urine.

Even though deriving from different microbial metabolic pathways, IS and PCS are both actively secreted by kidney and tightly bind to albumin when entering the circulation, in healthy individuals as well as in CKD patients [31]. IS and PCS are bound to albumin in a reversible manner for approximately 97% and 95%, respectively, so that a rapid equilibrium between the bound and free fraction is established [31]. They share the same albumin-binding site for which they are competitive binding inhibitors [21]. Because of their binding properties, IS and PCS cannot efficiently be removed by HD and accumulate in ESRD patients thus exacerbating their potential toxic effects, especially at the CV level [32].

Lots of studies suggested the toxicity of IS and PCS in different type of cells, especially in renal and endothelial cells, although a very interesting review by Vanholder advises to use caution when it comes to interpreting the results of cell studies and pay special attention to the use of realistic concentration of IS, PCS and albumin [20]. The most studied *in vitro* effect of protein-bound UTs is the induction of oxidative stress in endothelial cells. Dou *et al.* [33] first demonstrated that IS and p-cresol inhibit the endothelial proliferation in HUVEC and decrease endothelial wound repair. In the presence of albumin, at concentrations found in human plasma, IS and p-cresol decrease endothelial wound repair only at higher doses, comparable with the increase in the free non protein-bound fraction of these solutes in the experimental medium. This study suggests a role for these solutes in the endothelial dysfunction observed in uremic patients. The same authors found that IS induces a significant production of reactive oxygen species (ROS) in HUVEC [34], as confirmed by other researchers [22,31], increases NAD(P)H oxidase activity, and strongly decreases levels of glutathione, one of the most active antioxidant systems of the cells [34]. IS has been shown to induce a dose-dependent inhibition of endothelial cell proliferation [22] and an

increase in cell senescence through the ROS-NF- κ B-p53 pathway [22,35]. Furthermore, two studies [23,36] have investigated the effect of IS on oxidative stress and fibrosis in human proximal tubular cells, rat kidneys and heart. Their results suggest that IS (i) induces oxidative stress in renal cells and in CKD rats; (ii) worsens cardiac fibrosis and cardiomyocyte hypertrophy with enhanced oxidative stress and (iii) reduces anti-oxidative defence in hypertensive rats. In this regard, it is worthy pointing out that an *in vivo* study seems to confirm the involvement of IS in oxidative stress processes as it has been able to demonstrate the association of IS serum levels with the levels of pentosidine, a marker of carbonyl and oxidative stress, in a population of HD patients [37]. In addition, a previous *in vitro* study on cardiac cells had confirmed the aforementioned effects of IS on cardiac fibrosis. This research has highlighted a pro-fibrotic action of IS on rat cardiac fibroblast by stimulating collagen synthesis, and a pro-hypertrophic effect on rat cardiac myocyte via activation of MAPK and NF κ B pathways. Moreover, the authors have demonstrated that IS has pro-inflammatory effects on circulating human immune cells by stimulating cytokine mRNA expression via MAPK and NF κ B pathways. These experiments, though conducted in absence of albumin, seem to suggest a role for IS in cardiac remodelling as well as in the adverse effects on cardiac function [38]. As a further element, a negative relationship between IS and Klotho has been found in patients with CKD-associated left ventricular hypertrophy (LVH) [39]. In the same study the authors observed that, in normal mice, the intraperitoneal injection of IS induced LVH and renal Klotho downregulation, while Klotho inhibited IS-induced hypertrophy in cardiomyocytes by blocking oxidative stress, p38 and extracellular signal-regulated protein kinase 1/2 signalling pathways. These studies suggest that IS may contribute to the development of LVH in CKD [39]. Furthermore, IS and PCS decreased Klotho expression in renal tubules *in vitro* and *in vivo*, by oxidative stress-induced epigenetic silencing of the Klotho gene, as speculated by the authors [40].

Inflammation-associated tubule-interstitial injury is a common pathological mechanism for kidney injury: a recent study [41] has shown that PCS and IS are able to stimulate significant cellular inflammation and immune responses in mouse proximal renal tubular cell [41-43]. Accumulating evidence indicates that infiltrating leukocytes are important in the development of renal injury [44],

and that IS enhances leukocyte-endothelial interactions through up-regulation of E-selectin and augmentation of oxidative stress in both *in vitro* and *in vivo* models [45], thus proving its effect on endothelial inflammatory processes. IS has been shown to be able to induce, also in HK-2 proximal tubular cells, ROS production, the activation of NF- κ B/p53 and the upregulation of MCP-1 [46]; MCP-1 mRNA expression is also induced in rat kidneys [46] and endothelial cells [47]. MCP-1 plays an important role in recruiting monocytes/macrophages to injured tubule-interstitial tissue [48], so the increase in IS level in CKD patients might be involved in the development of tubule-interstitial injury through induction of MCP-1 in the kidneys [46].

In the same way, PCS has been shown to significantly increase the percentage of leucocytes displaying oxidative burst activity at baseline thus demonstrating the pro-inflammatory effects of this metabolite on unstimulated leucocytes [49]. Finally, IS has been observed to increase angiotensinogen expression in proximal tubular cells, which plays an important role in the development of CV damage [50].

Indole-3-acetic acid (IAA) is a protein-bound uremic toxin deriving, as IS, from the indole pathway of tryptophan microbial metabolism [51,52]. While showing high protein-binding ratios (94%), IAA has a higher reduction rates by HD (44%) compared to IS [31]. Literature data show that UTs derived from tryptophan, such as IS and IAA, bind the transcription factor aryl hydrocarbon receptor (AhR) whose activation is associated with an increase in CVD risk [53]. Indeed, the same group observed that IS and IAA induced an increased expression of tissue factor in endothelial and peripheral blood mononuclear cells, by AhR activation [54]. Moreover, they studied the pro-inflammatory and pro-oxidant effects of IAA in endothelial cells, finding an increased endothelial ROS production by IAA at uremic concentration, as well as the activation of pro-inflammatory enzyme cyclooxygenase-2 mediated by AhR [55]. IAA was also found to increase the endothelial expression of the inflammatory genes IL-6, IL-8, ICAM-1, and MCP-1 [55]. Furthermore, *in vitro* experiments have shown that IAA, like IS, induces the apoptosis of endothelial progenitors cells in CKD [56], although the causative mechanisms are still unknown. Interestingly, beside the potential role of AhR in the atherosclerotic process [57], this receptor could also be involved in the imbalance between vascular damage/regeneration in CKD [58]. However, when assessing IAA

actual toxicity, it is worth considering that the data reported are sometimes inconsistent [25] and need further insights.

A final remark about kynurenine, which derives from the main metabolic pathway of tryptophan, and its correlation with CKD severity and chronic inflammation [59]. The binding of kynurenine with AhR [60] is likely to be involved in the same events that lead to increased CV risk, as mentioned before [53]. Unlike IS and IAA, kynurenine is significantly reduced by HD [61].

How gut microbiota metabolites influence CV risk in CKD

The gut microbiota influences human physiology through its metabolites. The protein-bound UTs PCS, IS, IAA, but also other microbial metabolites such as Trimethylamine-N-Oxide (TMAO), Nitric Oxide (NO) and Short-Chain Fatty Acids (SCFA) represent the main molecules to which many experimental and clinical studies have ascribed a direct effect or an association with CV risk. They could be considered the mediators through which mainly the gut (but also the oral microbiota), influences CV risk in CKD. Table 2 summarizes the results of the main experimental and clinical studies that support the role of gut microbiota metabolites IS, PCS, TMAO and IAA in CV complications of CKD.

As already reported, the strong and evident pro-oxidative [31,36], pro-inflammatory [41] and pro-fibrotic effects [10-12,62] of IS and PCS shown in *in vitro* and *in vivo* experiments as well as in the *in vivo* induction of aortic calcification by IS [63] seem to provide a molecular basis for their role as co-promoters of CV and renal damage progression in CKD. In addition, it is worth considering that protein-bound UTs are not efficiently removed by dialytic treatment and that this contributes to additionally increased CV risk in ESRD [32,64]. Well-established research findings have ascribed adverse clinical outcomes to these UTs, as reported summarized in a recent meta-analysis which has investigated IS and PCS serum levels as biomarkers of CV events and all-cause mortality in CKD patients [24]. The authors of this study selected 11 out of 155 articles, with a total of 1,572 patients, using strict inclusion criteria and considering the risks of all-cause mortality and CV events as primary outcome measures. The results indicated that both elevated free PCS and IS were significantly associated with increased mortality in CKD patients, while elevated free PCS

levels, but not IS levels, were significantly associated with an increased risk of CV events in CKD patients [24]. The latest literature studies are mainly focused on dialysis patients and on the removal strategies of these protein-bound UTs. It can easily be inferred that the toxicity of elevated serum levels of IS and PCS is exacerbated in these patients [64]. Indeed, in a recent national multicenter prospective cohort study including 394 incident dialysis patients [64] the study of the free serum levels of 4 uremic metabolites (PCS, IS, hippurate and phenylacetylglutamine) has shown a correlation between higher PCS levels and a greater risk of CV mortality and first CV event. Moreover, patients with the highest levels of the four combined solutes had a greater risk of CV mortality. However, it is worthwhile reporting that a very recent study on a subgroup of 1,273 HD patients from the HEMO trial has revealed no association between IS, PCS, phenylacetylglutamine and hippurate and cardiac death, sudden cardiac death and first cardiovascular event [65], although the association between IS and PCS and higher risk of cardiac death and sudden cardiac death has been shown to be significant in patients with low serum albumin levels. There are two points, raised by the same authors that recommend some caution when interpreting these results: the first point being that in the study in question the total levels of PCS and IS were considered, while the free levels of UTs may be better indicators of their potential toxicity, as tissues are exposed to them. The second point is that, given the strong scientific rationale supporting the cardiotoxicity of IS and PCS, their role in uremic toxicity and CV risk cannot be excluded and that only a RCT could provide a strong demonstration [65].

TMAO is a downstream metabolite of dietary choline, phosphatidylcholine and L-carnitine, produced by the gut microbiota after their conversion into TMA and oxidized to TMAO in the liver [66]; high levels of its dietary precursors lead to an increase in TMAO circulating levels [67]. TMAO is normally cleared by the kidneys and, as a result, it is elevated in CKD patients [68]; unlike the other UTs, it has no high affinity with serum albumin, being efficiently removed by the dialytic treatment [69]. Accumulating evidence in the literature indicates a role for elevated TMAO levels as an indicator of increased risk of all-cause mortality in CKD patients [68]. Very recently, the group of Heianza has conducted a systematic review and meta-analysis of prospective studies on the association of TMAO and its precursors L-carnitine, choline and betaine with major acute

cardiovascular events (MACE), demonstrating that elevated levels of these metabolites are associated with MACE and all-cause mortality, independently of other traditional risk factors [66]. TMAO could modulate the CV risk by many potential mechanisms. An interesting study examined the gut microbiota-dependent metabolism of L-carnitine to produce TMAO in both rodents and humans, demonstrating a role for TMAO in atherosclerosis pathogenesis [67]. The authors showed that one pro-atherosclerotic mechanism observed for TMAO is the reduction of reverse cholesterol transport (RCT) through gut microbiota-dependent mechanisms *in vivo*, but the molecular mechanisms whereby TMAO inhibits RCT are not entirely clear [67]. Furthermore, other studies focused on the involvement of TMAO in atherosclerotic plaque development in animal models, showing a critical role for TMAO in increasing macrophage cholesterol accumulation and foam cell formation [70], enhancing human platelets hyper-reactivity, and enhancing *in vivo* thrombosis [71] or by a pro-inflammatory action [72].

As already mentioned, the microbial-derived metabolite IAA is another potential link with increased CV risk in CKD. Although less studied, its pro-inflammatory and pro-oxidant effects potentially explain the association with higher mortality and CV events observed in CKD patients; anyway, these effects remain to be explored *in vivo* [55].

Another microbial-dependent effect influencing CV risk is linked to the modulation of NO availability. Amongst its pleiotropic effects, NO acts as a paracrine vasodilator. It is an important regulator of vascular health and BP, and a reduced NO endogenous production results in endothelial dysfunction and increased CV risk [73]. NO is enzymatically produced by NO synthases in different cell types, particularly in endothelial cells, starting from L-arginine as precursor [74].

Alternatively, NO can be produced by the oral microbiota as a “back-up” system, namely the “enterosalivary nitrate-nitrite-NO pathway” [73]. Following intestinal absorption, dietary nitrate is concentrated in saliva and, by means of a chain of chemical reactions, is therefore reduced to nitrite by bacteria living in the mouth; a portion of the swallowed nitrate is further converted in the stomach into NO and other nitrogen species [74].

Dietary sources of nitrate include green leafy vegetables and beetroot [74]. Indeed, many trials explored the effects of orally administered nitrate on BP regulation and vascular function. They overall demonstrated beneficial and comparable effects of dietary NO sources (beetroot juice) or nitrate salts supplements on BP, vascular endothelial function and platelet aggregation, in both healthy conditions and in pathological contexts such as hypertension, diabetes, and CV risk [74,75]. On the other side, the observation that the use of antibacterial mouthwashes is accompanied by an elevation of systolic BP provides a demonstration of the important and physiological role of oral microbiota metabolism in maintaining vascular health [76]. Given that microbiota plays an important role in the nitrogen metabolism, an intriguing theory has been elaborated by Briskey and coworkers, suggesting that a balanced microbiota is essential to guarantee a fine regulation between the reduction/oxidation system in the human body and that its imbalance could contribute to CKD etiology [4]. The authors explain that nitrification and denitrification bacterial reactions, well studied in agricultural context, are largely unexplored in the context of human physiology, although they likely occur in the human body by oral and gut microbiota, and contribute to the regulation of the nitrogen cycle similarly to what happens in natural ecosystems. Oral microbiota composition, nitrate, nitrite, ammonia and oxygen concentrations are all factors influencing each other in a cause-consequence relationship still to be extensively studied, but certainly needing investigations.

Additionally, novel insights come from a suspected action of the gut microbiota in the BP regulation and its involvement in hypertension and salt sensitivity [77]. Like in gut-kidney axis, probably also in gut-heart axis a bidirectional relationship exists. Provided that the kidney plays the major role in BP regulation, some pioneering evidence suggests that hypertension is somehow linked and/or regulated by microbiota and its metabolites, although this research topic is quite new. Some human and animal studies indeed showed that hypertension and atherosclerosis associate with a decrease in microbiota diversity and richness, alteration in its composition and in the microbial metabolic profile [6]. This is a new field, comprising some studies carried on in animal models and a few association studies, while researches focusing on the mechanistic explanation of the microbiota effect on BP are currently lacking. Anyway, some hypotheses have been proposed. Gut

microbiota could control BP through the release of SCFA, end-products of saccharolytic fermentation of complex carbohydrates. Activation of different SCFA receptors present at renal level, such as Olfr78, Gpr41 or Gpr43, antagonistically results in raised or decreased BP [78]. In addition, another SCFA-mediated effect has been hypothesized by Santisteban *et al.*, acting on the modulation of the brain-gut-bone marrow axis, in which an enhanced sympathetic activity leads to hematopoietic stem cell mobilization, gut inflammation and raised BP [9]. Another link with CV system, still to be demonstrated, could be mediated by sulfur amino acids microbial metabolism leading to H₂S production, suspected to be linked to BP regulation [79].

Moreover, evidence from animal studies suggests that the gut microbiota could be involved in determining salt sensitivity, with a mechanism still to clarify but probably mediated again by SCFA [77]. Indeed, some murine studies showed differences in the fecal microbial composition between salt-sensitive (SS) and salt-resistant (SR) mice, and it was demonstrated that transplantation of SR cecal content to SS was able to confer a sustained increase in BP and a shorter lifespan, shedding a new light on the possibility that salt-sensitivity and hypertension could be transmissible by means of the gut microbiota [7,77].

The impact of nutritional management on CV risk

The protective effects of DASH diet or Mediterranean diet on the risk of incidence of CKD are well assessed in the literature [80,81]. The renal and CV protective nature of these two dietary patterns have been ascribed to the anti-proteinuric and anti-hypertensive effects mainly due to the limitation of salt intake and to the higher content of plant-based food [80-83].

In renal insufficiency, the total body sodium pool increases, causing hypertension and edema. This is due to the glomerular filtration rate (GFR) reduction, to hyperactivity of the sympathetic system and RAAS. A high salt intake increases the amount of urinary protein excretion [84], a major risk factor for the progression of both kidney and CV damage. Conversely, reducing salt intake reduces urine protein excretion by about 20% [85]. Salt restriction has been widely used in renal patients, often representing the only dietary intervention in the clinical practice. Low sodium diet is effective in lowering BP and proteinuria, and these effects are additive to the inhibition of RAAS and

diuretics [86]. On the contrary, unrestricted sodium diets impair and blunt the favorable effects of RAAS inhibitors on proteinuria and CKD progression, both in diabetic and in non-diabetic cohorts [87,88]. In advanced stages of CKD, salt restriction is mandatory to reduce the risk of fluid retention, and consequently of edema, heart failure or uncontrolled hypertension. So, consensus exists about the usefulness of salt restriction in CKD patients [89], although often difficult to achieve in clinical practice [90]. Instead, the history of plant-based dietary manipulation in CKD patients is quite various and often contradictory.

The DASH or Mediterranean diets are healthy dietary patterns proven to be useful as a prevention strategy for CKD in a population-based setting. Moreover, specific renal diets aimed at controlling the protein, sodium and phosphorus loads are needed in advanced stages of renal insufficiency. The nutritional treatment in CKD is necessary to slow down the pathology progression, to control its comorbidities and to prevent protein energy wasting [91,92]. The benefits of a controlled diet in CKD come from the reduction of the retention of UTs, sodium, phosphate and fixed acids [93], mainly derived by the catabolism of dietary proteins [94]. For this reason, nutritional treatments in renal patients have historically been focused on dietary protein restriction [91].

The new advances in our understanding of the central role of the gut microbiota in renal pathology and CV damage should be considered in the design of innovative renal diets, aimed at a new goal: modulating the gut microbiota, a non-traditional and modifiable risk factor. Dietary manipulation has indeed the potential to affect intestinal microbiota composition, metabolism and activity, and to control the CV risk by decreasing the production of the UTs described above.

Gastrointestinal bacteria can ferment complex carbohydrates (saccharolytic pathway) or use amino acids as an alternative fermentation substrate (proteolytic pathway): changes in the microbiota cause changes in metabolites production, which in turn can affect human pathophysiology [8]. In particular, an unbalance towards proteolytic metabolism may be deleterious. In this condition, an increase in the production of many toxic metabolites, namely amines, indoles, phenols, hydrogen sulfide occurs. These are the reasons why a gut is considered to be “healthy” when its metabolism is mainly saccharolytic. In a chronic renal failure setting, urea concentration increases in extracellular as well as in the intestinal fluids. This alters colonic pH and promotes the overgrowth

proteolytic microbial species, leading to an increased generation of UTs with a negative impact on CV risk [8]. In this context, the adoption of a targeted diet can shift the saccharolytic/proteolytic balance in a favorable way. Many plant-based food belonging to Mediterranean or DASH dietary schemes carry a considerable amount of fiber with prebiotic activity. This fiber remains undigested until the terminal gastrointestinal tract, where it selectively promotes the growth of probiotic, saccharolytic bacteria, leading to a decrease in protein-bound UTs [8,95,96].

The current nutritional guidelines in CKD do not take into consideration microbiota modulation. In fact, in order to control potassium intake, they often foresee a dramatic restriction of many fiber-rich foods such as fruits, vegetables and legumes, thus worsening potential constipation conditions. Actually, the risk of hyperkalemia is increased in CKD patients. This is due to the reduced GFR, though a very effective increase in K excretion by remaining nephrons occurs even in advanced CKD stages [97]. The main concern is the use of RAAS inhibitors, b-blockers, heparins that are frequently used, for their proven efficacy, in CKD and heart failure patients. In these settings, avoidance of high potassium intake is mandatory as a part of the strategy for hyperkalemia prevention. The upcoming new potassium binders may be a potential good option for less limitation of dietary potassium load, certainly to be further studied with randomized controlled trials aimed to define optimal management strategies [98].

But two aspects should be considered. First, vegetables contribute to an overall alkalizing effect: most fruit and vegetables are known to produce alkali when metabolized, contributing to acid neutralization [83,99]. Second, fiber intake has a positive effect on constipation. Constipation has recently been demonstrated to be a risk factor for CKD development and progression [100], for an unknown mechanism probably linked to uremic toxins retention and gut dysbiosis. This two effects of a plant-based diet can potentially and favorably affect potassium metabolism reducing the risk of hyperkalemia. Even in dialysis patients, the risk of hyperkalemia is linked to constipation and not to the amount of dietary potassium load [101]. Moreover, it is important to consider that phosphate from vegetable sources is poorly absorbed by the human intestine, thus limiting the effective body burden. So, in combination with protein restriction, that has already been demonstrated to be beneficial by reducing the proteolytic fermentation at colonic level, and beyond the expected

reduction in terms of onset and severity of uremic symptoms and complications [83,102,103], fiber intake should be encouraged in renal diets.

Moreover, the introduction of plant food results in beneficial effects also at CV levels: the DASH diet reduces BP at all sodium levels thanks to its content of vegetable matrices [104] and, as already reported above, an increased intake of green leafy vegetables and beetroot, rich in nitrate, favors oral microbiota-mediated NO generation [105]. This becomes particularly critical in HD patients, notoriously exposed to higher CV mortality. Here, the dialytic treatment *per se* seems to be responsible for a persistent depletion of circulating nitrite and nitrate, leading to a chronic reduction of steady-state NO levels [106]. Given the important action of their balance as an endothelium-independent redundant system for NO supply, and the experimental demonstration that their insufficient dietary intake promotes or accelerates disease, the role of nitrate and nitrite as dietary nutrients and not as harmful compounds, as they are currently considered, has been recently suggested for its benefits [75].

In conclusion, fiber intake provided by plant-based foods, combined with sodium and protein restriction should be encouraged in the dietary management of CKD. This kind of management would result in a combined favorable action at CV level through the modulation of glomerular hemodynamics, acid-base metabolism, effective phosphate load, lipid profile, systemic hypertension and NO generation, bowel transit, microbiota composition and metabolism, UTs levels, all affecting CV risk (Fig. 1).

Focus on microbiota - Perspectives: prevention and treatment

Prevention: lifestyle changes to prevent CKD pathologies by taking care of microbiota

There are some clues indicating that microbiota could play a role in the aetiology of CKD and hypertension [7]. Indeed, a dysbiotic microbiota could potentially represent a risk factor for the development of CKD after acute injury [5] or following a dysregulation of the nitrification/denitrification balance [4]. Moreover, reduced bowel transit and severe constipation have been associated with an increased risk of CVD, incident CKD/ESRD and progressive eGFR decline [100]. All these pieces of evidence encourage the adoption of preventative life-style

measures to keep a healthy and balanced microbiota, especially in the presence of other risk factors. Actually, the prevention could start earlier, before birth. There are proofs that low-birth weight and unbalanced nutrition in pregnant women account for risk factors for the development of CKD, CVD and metabolic disease in adult age [107]. In particular, since nephrogenesis is concentrated in the last trimester of pregnancy, pre-term birth and low-birth weight represent a risk factor both for developing CKD after an acute insult, and for hypertension, due to the low nephron number [107,108]. A major interaction and communication between different medical branches (gynaecology, neonatology and nephrology) in order to encourage a balanced nutrition during pregnancy, together with natural delivery and breastfeeding, beneficial for new-borns microbiota development, should be promoted as a preventative strategy for keeping under control the risk of developing CKD and hypertension [107].

Treatment: postbiotics and bacteriotherapy

The ability of the gut microbiota to respond to environmental changes offers the potentiality to act on it in order to control the progression of CKD and to reduce CV risk factors. The above dietary management could be complemented by the integrating a probiotic, prebiotic, synbiotic or the novel postbiotic approach. Indeed, some trials have tested the use of probiotics, prebiotics or synbiotics in CKD, reporting positive effects on uremic toxins, blood urea nitrogen and uric acid reduction, although none of these approaches has been demonstrated to be able to decrease UTs levels back to physiological ones [109]. Apart from this, the other limitations of these studies are the small sample size, the use of surrogate outcomes and the short study duration. It would be interesting to test on a longer timeframe if this approach could be effective in slowing down CKD progression and reducing CV events. In CVD context, the use of probiotics beverages and foods has been tested in some trials, demonstrating its efficacy in lowering systolic and in some case also diastolic BP, both in healthy and in hypertensive subjects [7], while the protective activity of prebiotics on hypertension and CV risk is mainly indirect, given the ability of some fiber-rich foods to improve the lipid and glycaemic frame [79,110]. Nevertheless, in a very recent study our group

has demonstrated an improvement in endothelial function measured by FMD after beta-glucans supplementation in healthy subjects [96].

A new perspective comes from the postbiotic approach, that is the possibility of intervention by nutritional supplementation with beneficial microbial end-products with a potent immunomodulating activity [111], especially in subjects with altered intestinal barrier integrity and ongoing intestinal inflammatory status [112]. Animal research has experimented the postbiotic approach as a safer alternative to antibiotics in poultry industry, with encouraging results on increased *in vitro* inhibitory activity against pathogens [113], chickens weight gain, feed efficiency, microbial composition, ileal inflammatory profile and volatile fatty acid production [114,115]. In humans, this approach is far from being applied in clinical studies, although there are promising perspectives in this regard. A pioneering study by Sokol *et al.* showed beneficial effects of oral administration of both *Faecalibacterium prausnitzii* or its supernatant in an animal model of Crohn's disease, in terms of correction of microbial dysbiosis and inflammation (both *in vivo* and *in vitro*, on epithelial and mononuclear cells) [111]. In a mouse model of obesity-induced insulin resistance, a muramyl-dipeptide derived from bacterial cell wall showed insulin-sensitizing effects [116], while a *Lactobacillus*-derived postbiotic showed efficacy in counteracting the inflammatory response associated to intestinal bowel disease or to *Salmonella* infection of healthy tissue [112]. Postbiotics have been suggested as a promising therapeutic option in children necrotizing enterocolitis [117]. Bacteria-derived peptides like Serine-Threonine peptide (STp) have been shown to restore *in vitro* a mature profile of gut dendritic cells in ulcerative colitis [118], while *Lactobacillus reuteri* DSM 17938 cell-free supernatant has been shown to modulate the gut dendritic cell phenotype and function [119]. Additionally, a *Lactobacillus fermentum* BGHV110 strain (HV110)-derived postbiotics have been able to stimulate *in vitro* a protective hepatic cells autophagy in response to high paracetamol doses [120]. The postbiotic approach has never been tested in CKD and certainly needs to be clinically validated. SCFAs, for example have the potential to exert their beneficial effects on colonic microenvironment, epithelial integrity, limitation of growth of potentially pathogenic species, and inflammation [121].

Bacteriotherapy or fecal microbiota transplant (FMT) is a therapeutic option by now validated only on recurrent *Clostridium difficile* infection and in course of validation in ulcerative colitis and metabolic syndrome [122]. There are some findings supporting the possibility to expand the field of application of this procedure to other clinical settings involving an alteration of the gut microbiota, including CKD and CV damage, but they would need extensive clinical research [123]. Contexts in which the mucosal immunity plays a key role in the aetiology and progression of the renal pathology and microbiota composition seem to be associated with pathology progression, such as IgAN [124-126] and lupus nephritis [127], could represent the ideal candidates for future FMT protocol settings [128]. The association between IgAN and the occurrence of inflammatory bowel disease [129] and the tuning of gut permeability and of the immune responses associated to lupus nephritis by microbiota manipulation [130] suggest that future efforts in this direction could be successful.

Conclusions

In conclusion, there is an important body of literature highlighting a role of the gut and oral microbiota in both aetiology and in progression of CKD, hypertension and CV damage. Certainly, the main gap to be filled in the literature concerns the elucidation of the underlying mechanisms and of the cause-effect relationships: is a dysbiotic microbiota one of the concurrent causal agents of hypertension, CV damage and progressive kidney failure or do these pathologies influence the composition and function of the microbiota, leading to dysbiosis? And, in both cases, what are the causal mechanism leading to the development of the diseases/dysbiosis? In the context of CKD some aspects have been clarified. It is by now well-established that the uremic state is responsible for an alteration of colonic environment, leading in turn to the modification of the microbiota composition and the increase in uremic toxin production that, together with the main mechanism, i.e. their reduced renal clearance, result in increased UTs circulating levels. However, the opposite relationship remains to be explored: if and how an altered microbiota can be responsible for inducing kidney loss of function.

Even so, research on the association between hypertension and a specific, dysbiotic microbial setting is still in its infancy and certainly needs to be expanded, even if there are some clues pointing towards a bidirectional communication of the gut-heart axis. In this field the need is felt to move from studies on animal models to human studies, and to switch from pure association studies to the investigation of the basal cause-effect relationships linking CKD, hypertension and microbiota.

Actually, in CKD the role of the microbiota in concurring to CV damage is emerging in the literature through a dual mechanism: firstly through altered function and secondly through increased production of harmful microbial metabolites. Importantly, microbiota and microbial derived UTs are emerging as potential modifiable risk factors of CV damage, provided that the combined action of diet and supplementation is capable of reducing UTs to the levels found in non-CKD subjects. So far no trial has been successful in this, albeit a reduction in UTs production rate is expected to be beneficial under any circumstances. In this regard, the microbiota represents a strategic factor to be considered in the clinical management of CKD patients.

Even so, new insights into the mechanistic aspects of the intricate pathophysiologic network of microbiota, kidney and heart will allow for designing specific, microbial-oriented therapies, to be integrated or associated to nutritional treatments. This combined approach could represent an effective strategy to i) postpone the need for dialysis, ii) support pre-emptive transplantation much more in the years to come, and iii) improve the life expectancy and the overall quality of life of our patients.

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Conflict of interests declaration

CC, MTR and LG declare no conflict of interests. AC received consultant honoraria from Shire Italia and from Vifor Fresenius Medical Care Renal Pharma Ltd.

References

1. Go AS, Chertow GM, Fan D et al. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med.* 2004 Sep 23;351(13):1296-305. doi: 10.1056/NEJMoa041031
2. Gansevoort RT, Correa-Rotter R, Hemmelgarn BR et al. Chronic kidney disease and cardiovascular risk: epidemiology, mechanisms, and prevention. *Lancet.* 2013 Jul 27;382(9889):339-52. doi: 10.1016/S0140-6736(13)60595-4
3. Kitai T, Kirsop J, Tang WH. Exploring the Microbiome in Heart Failure. *Curr Heart Fail Rep.* 2016 Apr;13(2):103-9. doi: 10.1007/s11897-016-0285-9
4. Briskey D, Tucker PS2, Johnson DW et al. Microbiota and the nitrogen cycle: Implications in the development and progression of CVD and CKD. *Nitric Oxide.* 2016 Jul 1;57:64-70. doi: 10.1016/j.niox.2016.05.002.
5. Ramezani A, Massy ZA, Meijers B et al. Role of the Gut Microbiome in Uremia: A Potential Therapeutic Target. *Am J Kidney Dis.* 2016 Mar;67(3):483-98. doi: 10.1053/j.ajkd.2015.09.027
6. Yang T, Santisteban MM, Rodriguez V et al. Gut dysbiosis is linked to hypertension. *Hypertension.* 2015 Jun;65(6):1331-40. doi: 10.1161/HYPERTENSIONAHA.115.05315
7. Galla S, Chakraborty S, Mell B et al. Microbiotal-Host Interactions and Hypertension. *Physiology (Bethesda).* 2017 May;32(3):224-233. doi: 10.1152/physiol.00003.2017
8. Montemurno E, Cosola C, Dalfino G et al. What would you like to eat, Mr CKD Microbiota? A Mediterranean Diet, please! *Kidney Blood Press Res.* 2014;39(2-3):114-23. doi: 10.1159/000355785

9. Santisteban MM, Kim S, Pepine CJ et al. Brain-gut-bone marrow axis: implications for hypertension and related therapeutics. *Circ Res* 118: 1327–1336, 2016.
doi:10.1161/CIRCRESAHA.116.307709
10. Miyazaki T, Ise M, Seo H et al. Indoxyl sulfate increases the gene expressions of TGF-beta 1, TIMP-1 and pro-alpha 1(I) collagen in uremic rat kidneys. *Kidney Int Suppl.* 1997 Nov;62:S15-22
11. Bolati D, Shimizu H, Higashiyama Y et al. Indoxyl sulfate induces epithelial-to-mesenchymal transition in rat kidneys and human proximal tubular cells. *Am J Nephrol.* 2011;34(4):318-23.
doi: 10.1159/000330852
12. Shimizu H, Yisireyli M, Nishijima F et al. Indoxyl sulfate enhances p53-TGF- β 1-Smad3 pathway in proximal tubular cells. *Am J Nephrol.* 2013;37(2):97-103. doi: 10.1159/000346420
13. Wu IW, Hsu KH, Lee CC et al. p-Cresyl sulphate and indoxyl sulphate predict progression of chronic kidney disease. *Nephrol Dial Transplant.* 2011 Mar;26(3):938-47. doi: 10.1093/ndt/gfq580
14. Lin CJ, Liu HL, Pan CF et al. Indoxyl sulfate predicts cardiovascular disease and renal function deterioration in advanced chronic kidney disease. *Arch Med Res.* 2012 Aug;43(6):451-6. doi: 10.1016/j.arcmed.2012.08.002
15. van den Brand JA, Mutsaers HA, van Zuilen AD et al. Uremic Solutes in Chronic Kidney Disease and Their Role in Progression. *PLoS One.* 2016 Dec 29;11(12):e0168117. doi: 10.1371/journal.pone.0168117
16. Vaziri ND, Wong J, Pahl M et al. Chronic kidney disease alters intestinal microbial flora. *Kidney Int.* 2013 Feb;83(2):308-15. doi: 10.1038/ki.2012.345
17. Cupisti A, D'Alessandro C, Gesualdo L et al. Non-Traditional Aspects of Renal Diets: Focus on Fiber, Alkali and Vitamin K1 Intake. *Nutrients.* 2017 Apr 29;9(5). pii: E444. doi: 10.3390/nu9050444
18. Hida M, Aiba Y, Sawamura S et al. Inhibition of the accumulation of uremic toxins in the blood and their precursors in the feces after oral administration of Lebenin, a lactic acid

- bacteria preparation, to uremic patients undergoing hemodialysis. *Nephron*. 1996; 74: 349–355. doi:10.1159/000189334
19. Wong J, Piceno YM, DeSantis TZ et al. Expansion of urease- and uricase-containing, indole- and p-cresol-forming and contraction of short-chain fatty acid-producing intestinal microbiota in ESRD. *Am J Nephrol*. 2014; 39(3):230-237. doi: 10.1159/000360010
 20. Vanholder R, Schepers E, Pletinck A et al. The Uremic Toxicity of Indoxyl Sulfate and p-Cresyl Sulfate: A Systematic Review. *J Am Soc Nephrol*. 2014;25(9):1897-1907. doi:10.1681/ASN.2013101062
 21. Meijers KI, Loo H De, Bammens B et al. P-Cresyl Sulfate and Indoxyl Sulfate in Hemodialysis Patients. *Clin J Am Soc Nephrol*. 2009 Dec;4(12):1932-8. doi: 10.2215/CJN.02940509
 22. Yu M, Kim YJ, Kang D-H. Indoxyl Sulfate-Induced Endothelial Dysfunction in Patients with Chronic Kidney Disease via an Induction of Oxidative Stress. *Clin J Am Soc Nephrol*. 2011;6(1):30-39. doi: 10.2215/CJN.05340610
 23. Yisireyili M, Shimizu H, Saito S et al. Indoxyl sulfate promotes cardiac fibrosis with enhanced oxidative stress in hypertensive rats. *Life Sci*. 2013;92(24-26):1180-1185. doi: 10.1016/j.lfs.2013.05.008
 24. Lin CJ, Wu V, Wu PC et al. Meta-analysis of the associations of p-cresyl sulfate (PCS) and indoxyl sulfate (IS) with cardiovascular events and all-cause mortality in patients with chronic renal failure. *PLoS One*. 2015;10(7):1-14. doi:10.1371/journal.pone.0132589
 25. Aronov PA, Luo FJ, Plummer NS et al. Colonic contribution to uremic solutes. *J Am Soc Nephrol*. 2011; 22(9):1769-76. doi: 10.1681/ASN.2010121220
 26. Pasternack A, Kuhlback B, Tallgren Lg. Serum Indican in Renal Disease. *Acta Medica Scandinavica*. 1964, Vol. 176, fasc. 6, 751-756
 27. Wikoff WR, Nagle MA, Kouznetsova VL et al. Untargeted metabolomics identifies enterobiome metabolites and putative uremic toxins as substrates of organic anion transporter 1 (Oat1). *J Proteome Res*. 2011 Jun 3;10(6):2842-51. doi: 10.1021/pr200093w

28. de Loor H, Bammens B, Evenepoel P, De Preter V, Verbeke K. Gas Chromatographic-Mass Spectrometric Analysis for Measurement of p-Cresol and Its Conjugated Metabolites in Uremic and Normal Serum. *Clinical Chemistry* 2005, 51, No. 8, 1535-1538. doi: 10.1373/clinchem.2005.050781
29. Wengle B, Hellström K. Volatile phenols in serum of uraemic patients. *Clinical Science* (1972) 43, 493-498
30. Gryp T, Vanholder R, Vaneechoutte M et al. p-Cresyl Sulfate. *Toxins* (Basel). 2017 Jan 29;9(2). pii: E52. doi: 10.3390/toxins9020052
31. Itoh Y, Ezawa A, Kikuchi K et al. Protein-bound uremic toxins in hemodialysis patients measured by liquid chromatography/tandem mass spectrometry and their effects on endothelial ROS production. *Anal Bioanal Chem*. 2012;403(7):1841-1850. doi:10.1007/s00216-012-5929-3
32. Sirich TL, Funk BA, Plummer NS et al. Prominent Accumulation in Hemodialysis Patients of Solutes Normally Cleared by Tubular Secretion. *J Am Soc Nephrol*. 2014 Mar;25(3):615-22. doi: 10.1681/ASN.2013060597
33. Dou L, Bertrand E, Cerini C, et al. The uremic solutes p-cresol and indoxyl sulfate inhibit endothelial proliferation and wound repair. *Kidney Int*. 2004;65(2):442-451. doi:10.1111/j.1523-1755.2004.00399.x
34. Dou L, Jourde-Chiche N, Faure V et al. The uremic solute indoxyl sulfate induces oxidative stress in endothelial cells. *J Thromb Haemost*. 2007 Jun;5(6):1302-8. doi: 10.1111/j.1538-7836.2007.02540.x
35. Shimizu H, Bolati D, Adijiang A et al. NF- κ B plays an important role in indoxyl sulfate-induced cellular senescence, fibrotic gene expression, and inhibition of proliferation in proximal tubular cells. *Am J Physiol Cell Physiol*. 2011 Nov;301(5):C1201-12. doi: 10.1152/ajpcell.00471.2010
36. Bolati D, Shimizu H, Yisireyili M et al. Indoxyl sulfate, a uremic toxin, downregulates renal expression of Nrf2 through activation of NF- κ B. *BMC Nephrol*. 2013;14(1):1. doi:10.1186/1471-2369-14-56

37. Kato A, Odamaki M, Hishida A. Association between blood indoxyl sulfate and carbonyl stress marker in hemodialysis patients. *Clin Nephrol*. 2003 Sep;60(3):161-7. doi: 10.2215/CJN.04730514
38. Lekawanvijit S, Adrahtas A, Kelly DJ et al. Does indoxyl sulfate, a uraemic toxin, have direct effects on cardiac fibroblasts and myocytes? *Eur Heart J*. 2010;31(14):1771-9. doi: 10.1093/eurheartj/ehp574
39. Yang K, Wang C, Nie L et al. Klotho Protects Against Indoxyl Sulphate-Induced Myocardial Hypertrophy. *J Am Soc Nephrol* 2015; 26(10):2434-46. doi: 10.1681/ASN.2014060543
40. Sun CY, Chang SC, Wu MS. Suppression of Klotho expression by protein-bound uremic toxins is associated with increased DNA methyltransferase expression and DNA hypermethylation. *Kidney Int* 2012;81(7):640-50. doi:10.1038/ki.2011.445
41. Sun CY, Hsu HH, Wu MS. P-Cresol sulfate and indoxyl sulfate induce similar cellular inflammatory gene expressions in cultured proximal renal tubular cells. *Nephrol Dial Transplant*. 2013;28(1):70-78. doi: 10.1093/ndt/gfs133
42. Tonelli M, Sacks F, Pfeffer M et al. Biomarkers of inflammation and progression of chronic kidney disease. *Kidney Int*. 2005 Jul;68(1):237-45. doi: 10.1111/j.1523-1755.2005.00398.x
43. Lee SB, Kalluri R. Mechanistic connection between inflammation and fibrosis. *Kidney Int Suppl*. 2010 Dec;(119):S22-6. doi: 10.1038/ki.2010.418
44. Holdsworth SR, Tipping PG. Leukocytes in glomerular injury. *Holdsworth Semin Immunopathol*. 2007 Nov;29(4):355-74
45. Ito S, Osaka M, Higuchi Y et al. Indoxyl sulfate induces leukocyte-endothelial interactions through up-regulation of E-selectin. *J Biol Chem*. 2010 Dec 10;285(50):38869-75. doi: 10.1074/jbc.M110.166686
46. Shimizu H, Bolati D, Higashiyama Y et al. Indoxyl sulfate upregulates renal expression of MCP-1 via production of ROS and activation of NF- κ B, p53, ERK, and JNK in proximal tubular cells. *Life Sci*. 2012;90(13-14):525-530. doi:10.1016/j.lfs.2012.01.013

47. Tumor Z, Shimizu H, Enomoto A et al. Indoxyl sulfate upregulates expression of ICAM-1 and MCP-1 by oxidative stress-induced NF- κ B activation. *Am J Nephrol* 2010;31:435–41. doi: 10.1159/000299798
48. Viedt C, Dechend R, Fei J et al. MCP-1 induces inflammatory activation of human tubular epithelial cells: involvement of the transcription factors, nuclear factor-kappaB and activating protein-1. *J Am Soc Nephrol* 2002;13: 1534–47. doi: 10.1097/01.ASN.0000015609.31253.7F
49. Schepers E, Meert N, Glorieux G et al. P-cresylsulphate, the main in vivo metabolite of p-cresol, activates leucocyte free radical production. *Nephrol Dial Transplant*. 2007 Feb;22(2):592-6. doi: 10.1093/ndt/gfl584
50. Shimizu H, Saito S, Higashiyama Y et al. CREB, NF- κ B, and NADPH oxidase coordinately upregulate indoxyl sulfate-induced angiotensinogen expression in proximal tubular cells. *Am J Physiol Cell Physiol*. 2013 Apr 1;304(7):C685-92. doi: 10.1152/ajpcell.00236.2012
51. Koga J, Syono K, Ichikawa T et al. Involvement of L-tryptophan aminotransferase in indole-3-acetic acid biosynthesis in *Enterobacter cloacae*. *Biochim. Biophys. Acta* 1994, 1209, 241–247. doi: 10.1016/0167-4838(94)90191-0
52. Zhu W, Stevens AP, Dettmer K et al. Quantitative profiling of tryptophan metabolites in serum, urine, and cell culture supernatants by liquid chromatography-tandem mass spectrometry. *Anal. Bioanal. Chem.* 2011, 401, 3249–3261. doi: 10.1007/s00216-011-5436-y
53. Sallée M, Dou L, Cerini C et al. The Aryl Hydrocarbon Receptor-Activating Effect of Uremic Toxins from Tryptophan Metabolism: A New Concept to Understand Cardiovascular Complications of Chronic Kidney Disease. *Toxins* 2014, 6, 934-949; doi:10.3390/toxins6030934
54. Gondouin B, Cerini C, Dou L et al. Indolic uremic solutes increase tissue factor production in endothelial cells by the aryl hydrocarbon receptor pathway. *Kidney Int.* 2013. 84, 733–744; doi:10.1038/ki.2013.133
55. Dou L, Sallée M, Cerini C et al. The cardiovascular effect of the uremic solute indole-3 acetic acid. *J Am Soc Nephrol.* 2015 Apr;26(4):876-87. doi: 10.1681/ASN.2013121283

56. Jourde-Chiche N, Dou L, Sabatier F et al. Levels of circulating endothelial progenitor cells are related to uremic toxins and vascular injury in HD patients. *J Thromb Haemost*. 2009;7(9):1576-84. doi: 10.1111/j.1538-7836.2009.03540.x
57. Wu D, Nishimura N, Kuo V et al. Activation of aryl hydrocarbon receptor induces vascular inflammation and promotes atherosclerosis in apolipoprotein e^{-/-} mice. *Arterioscler Thromb Vasc Biol*. 2011; 31: 1260–1267. doi: 10.1161/ATVBAHA.110.220202
58. Boitano AE, Wang J, Romeo R et al. Aryl hydrocarbon receptor antagonists promote the expansion of human hematopoietic stem cells. *Science*. 2010; 329: 1345–1348. doi: 10.1126/science.1191536
59. Schefold JC, Zeden JP, Fotopoulou C et al. Increased indoleamine 2,3-dioxygenase (IDO) activity and elevated serum levels of tryptophan catabolites in patients with chronic kidney disease: A possible link between chronic inflammation and uraemic symptoms. *Nephrol Dial Transplant*. 2009, 24, 1901–1908. doi: 10.1093/ndt/gfn739
60. DiNatale BC, Murray IA, Schroeder JC et al. Kynurenic acid is a potent endogenous aryl hydrocarbon receptor ligand that synergistically induces interleukin-6 in the presence of inflammatory signaling. *Toxicol. Sci*. 2010, 115, 89–97. doi: 10.1093/toxsci/kfq024
61. Pawlak D, Pawlak K, Malyszko J et al. Accumulation of toxic products degradation of kynurenine in hemodialyzed patients. *Int. Urol. Nephrol*. 2001, 33, 399–404
62. Sun CY, Chang SC, Wu MS. Uremic toxins induce kidney fibrosis by activating intrarenal renin-angiotensin-aldosterone system associated epithelial-to-mesenchymal transition. *PLoS One*. 2012;7(3):e34026. doi: 10.1371/journal.pone.0034026
63. Adijiang A, Goto S, Uramoto S et al. Indoxyl sulphate promotes aortic calcification with expression of osteoblast-specific proteins in hypertensive rats. *Nephrol Dial Transplant*. 2008 Jun;23(6):1892-901. doi: 10.1093/ndt/gfm861
64. Shafi T, Meyer TW, Hostetter TH et al. Free levels of selected organic solutes and cardiovascular morbidity and mortality in hemodialysis patients: Results from the retained organic solutes and clinical outcomes (ROSCO) investigators. *PLoS One*. 2015;10(5):1-17. doi:10.1371/journal.pone.0126048

65. Shafi T, Sirich TL, Meyer TW et al. Results of the HEMO Study suggest that p-cresol sulfate and indoxyl sulfate are not associated with cardiovascular outcomes. *Kidney Int.* 2017 Dec;92(6):1484-1492. doi: 10.1016/j.kint.2017.05.012
66. Heianza Y, Ma W2, Manson JE et al. Gut Microbiota Metabolites and Risk of Major Adverse Cardiovascular Disease Events and Death: A Systematic Review and Meta-Analysis of Prospective Studies. *J Am Heart Assoc.* 2017 Jun 29;6(7). pii: e004947. doi: 10.1161/JAHA.116.004947
67. Koeth RA, Wang Z, Levison BS et al. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nat Med.* 2013 May;19(5):576-85. doi: 10.1038/nm.3145
68. Tang WH, Wang Z, Kennedy DJ et al. Gut microbiota-dependent trimethylamine N-oxide (TMAO) pathway contributes to both development of renal insufficiency and mortality risk in chronic kidney disease. *Circ Res.* 2015 Jan 30;116(3):448-55. doi: 10.1161/CIRCRESAHA.116.305360
69. Bain MA, Faull R, Fornasini G et al. Accumulation of trimethylamine and trimethylamine-N-oxide in end-stage renal disease patients undergoing haemodialysis. *Nephrol Dial Transplant.* 2006 May;21(5):1300-4. doi: 10.1093/ndt/gfk056
70. Wang Z, Klipfell E, Bennett BJ et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature.* 2011 Apr 7;472(7341):57-63. doi: 10.1038/nature09922
71. Zhu W, Gregory JC, Org E et al. Gut Microbial Metabolite TMAO Enhances Platelet Hyperreactivity and Thrombosis Risk. *Cell.* 2016 Mar 24;165(1):111-24. doi: 10.1016/j.cell.2016.02.011
72. Seldin MM, Meng Y, Qi H et al. Trimethylamine N-Oxide Promotes Vascular Inflammation Through Signaling of Mitogen-Activated Protein Kinase and Nuclear Factor- κ B. *J Am Heart Assoc.* 2016 Feb 22;5(2). pii: e002767. doi: 10.1161/JAHA.115.002767
73. Bryan NS, Tribble G, Angelov N et al. Oral Microbiome and Nitric Oxide: the Missing Link in the Management of Blood Pressure. *Curr Hypertens Rep.* 2017 Apr;19(4):33. doi: 10.1007/s11906-017-0725-2.

74. Hezel MP, Weitzberg E. The oral microbiome and nitric oxide homeostasis. *Oral Dis.* 2015 Jan;21(1):7-16. doi: 10.1111/odi.12157
75. Bryan NS, Ivy JL. Inorganic nitrite and nitrate: evidence to support consideration as dietary nutrients. *Nutr Res.* 2015 Aug;35(8):643-54. doi: 10.1016/j.nutres.2015.06.001
76. Bondonno CP, Liu AH, Croft KD et al. Antibacterial mouthwash blunts oral nitrate reduction and increases blood pressure in treated hypertensive men and women. *Am J Hypertens.* 2015 May;28(5):572-5. doi: 10.1093/ajh/hpu192
77. Mell B, Jala VR, Mathew AV et al. Evidence for a link between gut microbiota and hypertension in the Dahl rat. *Physiol Genomics.* 2015 Jun;47(6):187-97. doi: 10.1152/physiolgenomics.00136.2014
78. Pluznick JL, Protzko RJ, Gevorgyan H et al. Olfactory receptor responding to gut microbiota-derived signals plays a role in renin secretion and blood pressure regulation. *Proc Natl Acad Sci U S A.* 2013 Mar 12;110(11):4410-5. doi: 10.1073/pnas.1215927110
79. Al Khodor S, Reichert B, Shatat IF. The Microbiome and Blood Pressure: Can Microbes Regulate Our Blood Pressure? *Front Pediatr.* 2017 Jun 19;5:138. doi: 10.3389/fped.2017.00138
80. Rebholz CM, Crews DC, Grams ME et al. DASH (Dietary Approaches to Stop Hypertension) Diet and Risk of Subsequent Kidney Disease. *Am J Kidney Dis.* 2016 Dec;68(6):853-861. doi: 10.1053/j.ajkd.2016.05.019
81. Asghari G, Farhadnejad H, Mirmiran P et al. Adherence to the Mediterranean diet is associated with reduced risk of incident chronic kidney diseases among Tehranian adults. *Hypertens Res.* 2017 Jan;40(1):96-102. doi: 10.1038/hr.2016.98
82. Gallieni M, Cupisti A. DASH and Mediterranean Diets as Nutritional Interventions for CKD Patients. *Am J Kidney Dis.* 2016 Dec;68(6):828-830. doi: 10.1053/j.ajkd.2016.09.001.
83. Di Iorio BR, Marzocco S, Bellasi A et al. Nutritional therapy reduces protein carbamylation through urea lowering in chronic kidney disease. *Nephrol Dial Transplant* 2017, 1-10. doi: 10.1093/ndt/gfx203

84. Verhave JC, Hillege HL, Burgerhof J et al. Sodium intake affects urinary albumin excretion especially in overweight subjects. *J Intern Med.* 2004 Oct;256(4):324-30. doi: 10.1111/j.1365-2796.2004.01390.x
85. He FJ, Marciniak M, Visagie E et al. Effect of modest salt reduction on blood pressure, urinary albumin, and pulse wave velocity in white, black, and Asian mild hypertensives. *Hypertension.* 2009 Sep;54(3):482-8. doi: 10.1161/HYPERTENSIONAHA.109.133223
86. Vogt L, Waanders F, Boomsma F et al. Effects of dietary sodium and hydrochlorothiazide on the antiproteinuric efficacy of losartan. *J Am Soc Nephrol.* 2008 May;19(5):999-1007. doi: 10.1681/ASN.2007060693
87. Lambers Heerspink HJ, Holtkamp FA, Parving HH et al. Moderation of dietary sodium potentiates the renal and cardiovascular protective effects of angiotensin receptor blockers. *Kidney Int.* 2012 Aug;82(3):330-7. doi: 10.1038/ki.2012.74
88. Vegter S, Perna A, Postma MJ et al. Sodium intake, ACE inhibition, and progression to ESRD. *J Am Soc Nephrol.* 2012 Jan;23(1):165-73. doi: 10.1681/ASN.2011040430
89. Bellizzi V, Bianchi S, Bolasco P, Brunori G, Cupisti A, Gambaro G, Gesualdo L, Polito P, Santoro D, Santoro A. A Delphi consensus panel on nutritional therapy in chronic kidney disease. *J Nephrol.* 2016 Oct;29(5):593-602
90. de Borst MH, Navis G. Sodium intake, RAAS-blockade and progressive renal disease. *Pharmacol Res.* 2016 May;107:344-351. doi: 10.1016/j.phrs.2016.03.037
91. Aparicio M, Bellizzi V, Chauveau P et al. Keto acid therapy in predialysis chronic kidney disease patients: final consensus. *J Ren Nutr.* 2012 Mar;22(2 Suppl):S22-4. doi: 10.1053/j.jrn.2011.09.006
92. Kovesdy CP, Kalantar-Zadeh K. Back to the future: restricted protein intake for conservative management of CKD, triple goals of renoprotection, uremia mitigation, and nutritional health. *Int Urol Nephrol.* 2016 May;48(5):725-9. doi: 10.1007/s11255-016-1224-0
93. D'Alessandro C, Piccoli GB, Calella P, Brunori G, Pasticci F, Egidi MF, Capizzi I, Bellizzi V, Cupisti A. "Dietaly": practical issues for the nutritional management of CKD patients in Italy. *BMC Nephrol.* 2016 Jul 29;17(1):102

94. Mitch WE, Remuzzi G. Diets for patients with chronic kidney disease, should we reconsider? *BMC Nephrol.* 2016 Jul 11;17(1):80. doi: 10.1186/s12882-016-0283-x
95. De Angelis M, Montemurno E, Vannini L et al. Effect of Whole-Grain Barley on the Human Fecal Microbiota and Metabolome. *Appl Environ Microbiol.* 2015 Nov;81(22):7945-56. doi: 10.1128/AEM.02507-15
96. Cosola C, De Angelis M, Rocchetti MT et al. Beta-Glucans Supplementation Associates with Reduction in P-Cresyl Sulfate Levels and Improved Endothelial Vascular Reactivity in Healthy Individuals. *PLoS One.* 2017 Jan 20;12(1):e0169635. doi: 10.1371/journal.pone.0169635
97. Kovesdy CP. Management of hyperkalaemia in chronic kidney disease. *Nat Rev Nephrol.* 2014 Nov;10(11):653-62. doi: 10.1038/nrneph.2014.168
98. Rossignol P, Legrand M, Kosiborod M et al. Emergency management of severe hyperkalemia: Guideline for best practice and opportunities for the future. *Pharmacol Res.* 2016 Nov;113(Pt A):585-591. doi: 10.1016/j.phrs.2016.09.039
99. Goraya N, Wesson DE. Dietary management of chronic kidney disease: protein restriction and beyond. *Curr Opin Nephrol Hypertens.* 2012 Nov;21(6):635-40. doi: 10.1097/MNH.0b013e328357a69b
100. Sumida K, Molnar MZ, Potukuchi PK et al. Constipation and Incident CKD. *J Am Soc Nephrol.* 2017 Apr;28(4):1248-1258. doi: 10.1681/ASN.2016060656
101. St-Jules DE, Goldfarb DS, Sevick MA. Nutrient Non-equivalence: Does Restricting High-Potassium Plant Foods Help to Prevent Hyperkalemia in Hemodialysis Patients? *J Ren Nutr.* 2016 Sep;26(5):282-7. doi: 10.1053/j.jrn.2016.02.005
102. Niwa T, Tsukushi S, Ise M et al. Indoxyl sulfate and progression of renal failure: effects of a low-protein diet and oral sorbent on indoxyl sulfate production in uremic rats and undialyzed uremic patients. *Miner Electrolyte Metab.* 1997;23(3-6):179-84
103. Marzocco S, Dal Piaz F, Di Micco L et al. Very low protein diet reduces indoxyl sulfate levels in chronic kidney disease. *Blood Purif.* 2013; 35(1-3):196-201. doi: 10.1159/000346628

104. Sacks FM, Svetkey LP, Vollmer WM et al. Effects on blood pressure of reduced dietary sodium and the Dietary Approaches to Stop Hypertension (DASH) diet. DASH-Sodium Collaborative Research Group. *N Engl J Med.* 2001; 344(1): 3-10. doi: 10.1056/NEJM200101043440101
105. Weitzberg E, Lundberg JO. Novel aspects of dietary nitrate and human health. *Annu Rev Nutr.* 2013;33:129-59. doi: 10.1146/annurev-nutr-071812-161159
106. Bryan NS, Torregrossa AC, Mian AI et al. Acute effects of hemodialysis on nitrite and nitrate: potential cardiovascular implications in dialysis patients. *Free Radic Biol Med.* 2013 May;58:46-51. doi: 10.1016/j.freeradbiomed.2013.01.020
107. Luyckx VA, Perico N, Somaschini M et al. A developmental approach to the prevention of hypertension and kidney disease: a report from the Low Birth Weight and Nephron Number Working Group. *Lancet.* 2017 Jul 22;390(10092):424-428. doi: 10.1016/S0140-6736(17)30576-7
108. Brenner BM, Garcia DL, Anderson S. Glomeruli and blood pressure. Less of one, more the other? *Am J Hypertens.* 1988 Oct;1(4 Pt 1):335-47
109. Sabatino A, Regolisti G, Cosola C et al. Intestinal Microbiota in Type 2 Diabetes and Chronic Kidney Disease. *Curr Diab Rep.* 2017 Mar;17(3):16. doi: 10.1007/s11892-017-0841-z
110. Andersson KE, Hellstrand P. Dietary oats and modulation of atherogenic pathways. *Mol Nutr Food Res.* 2012 Jul;56(7):1003-13. doi: 10.1002/mnfr.201100706
111. Sokol H, Pigneur B, Watterlot L et al. Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proc Natl Acad Sci U S A.* 2008 Oct 28;105(43):16731-6. doi: 10.1073/pnas.0804812105
112. Tsilingiri K, Barbosa T, Penna G et al. Probiotic and postbiotic activity in health and disease: comparison on a novel polarised ex-vivo organ culture model. *Gut.* 2012 Jul;61(7):1007-15. doi: 10.1136/gutjnl-2011-300971
113. Kareem KY, Hooi Ling F, Teck Chwen et al. Inhibitory activity of postbiotic produced by strains of *Lactobacillus plantarum* using reconstituted media supplemented with inulin. *Gut Pathog.* 2014 Jun 14;6:23. doi: 10.1186/1757-4749-6-23

114. Kareem KY, Loh TC, Foo HL et al. Effects of dietary postbiotic and inulin on growth performance, IGF1 and GHR mRNA expression, faecal microbiota and volatile fatty acids in broilers. *BMC Vet Res.* 2016 Aug 5;12(1):163. doi: 10.1186/s12917-016-0790-9
115. Kareem KY, Loh TC, Foo HL et al. Influence of postbiotic RG14 and inulin combination on cecal microbiota, organic acid concentration, and cytokine expression in broiler chickens. *Poult Sci.* 2017 Apr 1;96(4):966-975. doi: 10.3382/ps/pew362
116. Cavallari JF, Fullerton MD, Duggan BM et al. Muramyl Dipeptide-Based Postbiotics Mitigate Obesity-Induced Insulin Resistance via IRF4. *Cell Metab.* 2017 May 2;25(5):1063-1074.e3. doi: 10.1016/j.cmet.2017.03.021
117. Patel RM, Denning PW. Therapeutic use of prebiotics, probiotics, and postbiotics to prevent necrotizing enterocolitis: what is the current evidence? *Clin Perinatol.* 2013 Mar;40(1):11-25. doi: 10.1016/j.clp.2012.12.002
118. Al-Hassi HO, Mann ER, Sanchez B et al. Altered human gut dendritic cell properties in ulcerative colitis are reversed by *Lactobacillus plantarum* extracellular encrypted peptide STp. *Mol Nutr Food Res.* 2014 May;58(5):1132-43. doi: 10.1002/mnfr.201300596
119. Haileselassie Y, Navis M, Vu N et al. Postbiotic Modulation of Retinoic Acid Imprinted Mucosal-like Dendritic Cells by Probiotic *Lactobacillus reuteri* 17938 In Vitro. *Front Immunol.* 2016 Mar 17;7:96. doi: 10.3389/fimmu.2016.00096
120. Dinić M, Lukić J, Djokić J et al. *Lactobacillus fermentum* Postbiotic-induced Autophagy as Potential Approach for Treatment of Acetaminophen Hepatotoxicity. *Front Microbiol.* 2017 Apr 6;8:594. doi: 10.3389/fmicb.2017.00594
121. Puertollano E, Kolida S, Yaqoob P. Biological significance of short-chain fatty acid metabolism by the intestinal microbiome. *Curr Opin Clin Nutr Metab Care.* 2014 Mar;17(2):139-44. doi: 10.1097/MCO.0000000000000025
122. Cammarota G, Ianiro G, Tilg H et al. on behalf of European FMT Working Group. European consensus conference on faecal microbiota transplantation in clinical practice. *Gut.* 2017 Apr;66(4):569-580. doi: 10.1136/gutjnl-2016-313017

123. Chanyi RM, Craven L, Harvey B et al. Faecal microbiota transplantation: Where did it start? What have studies taught us? Where is it going? *SAGE Open Med.* 2017 May 11;5:2050312117708712. doi: 10.1177/2050312117708712
124. McCarthy DD, Kujawa J, Wilson C. Mice overexpressing BAFF develop a commensal flora-dependent, IgA-associated nephropathy. *J Clin Invest.* 2011 Oct;121(10):3991-4002. doi: 10.1172/JCI45563
125. De Angelis M, Montemurno E, Piccolo M et al. Microbiota and metabolome associated with immunoglobulin A nephropathy (IgAN). *PLoS One.* 2014 Jun 12;9(6):e99006. doi: 10.1371/journal.pone.0099006
126. Piccolo M, De Angelis M, Lauriero G et al. Salivary Microbiota Associated with Immunoglobulin A Nephropathy. *Microb Ecol.* 2015 Aug;70(2):557-65. doi: 10.1007/s00248-015-0592-9
127. Sánchez B, Hevia A, González S et al. Interaction of Intestinal Microorganisms with the Human Host in the Framework of Autoimmune Diseases. *Front Immunol.* 2015 Nov 20;6:594. doi: 10.3389/fimmu.2015.00594
128. Cohen NA, Maharshak N. Novel Indications for Fecal Microbial Transplantation: Update and Review of the Literature. *Dig Dis Sci.* 2017 May;62(5):1131-1145. doi: 10.1007/s10620-017-4535-9
129. Coppo R. The intestine-renal connection in IgA nephropathy. *Nephrol Dial Transplant.* 2015 Mar;30(3):360-6. doi: 10.1093/ndt/gfu343
130. Mu Q, Zhang H^{2,3}, Liao X et al. Control of lupus nephritis by changes of gut microbiota. *Microbiome.* 2017 Jul 11;5(1):73. doi: 10.1186/s40168-017-0300-8
131. Masereeuw R, Mutsaers HAM, Toyohara T et al. The Kidney and Uremic Toxin Removal: Glomerulus or Tubule? *Seminars in Nephrology.* 2014; 34(2):191–208. doi: 10.1016/j.semnephrol.2014.02.010
132. Teft WA, Morse BL, Leake BF et al. Identification and Characterization of Trimethylamine-N-oxide Uptake and Efflux Transporters. *Mol Pharm.* 2017 Jan 3;14(1):310-318. doi: 10.1021/acs.molpharmaceut.6b00937

Figure 1. Potential benefits of nutritional and supplementation approaches targeting microbiota in CKD patients. In CKD, nutritional management and supplementation, including salt and protein restriction, vegetable intake, and the use of pro-, pre- and synbiotics, has a variety of potential benefits. These comprise: modulation of gut microbiota dysbiosis and decreased colonic production of proteolytic-derived UTs, decreased circulating levels of blood urea nitrogen and uric acid, reduction of inflammation and oxidative stress, amelioration of bowel transit, glucose and lipid metabolism, acid-base balance and hypertension.

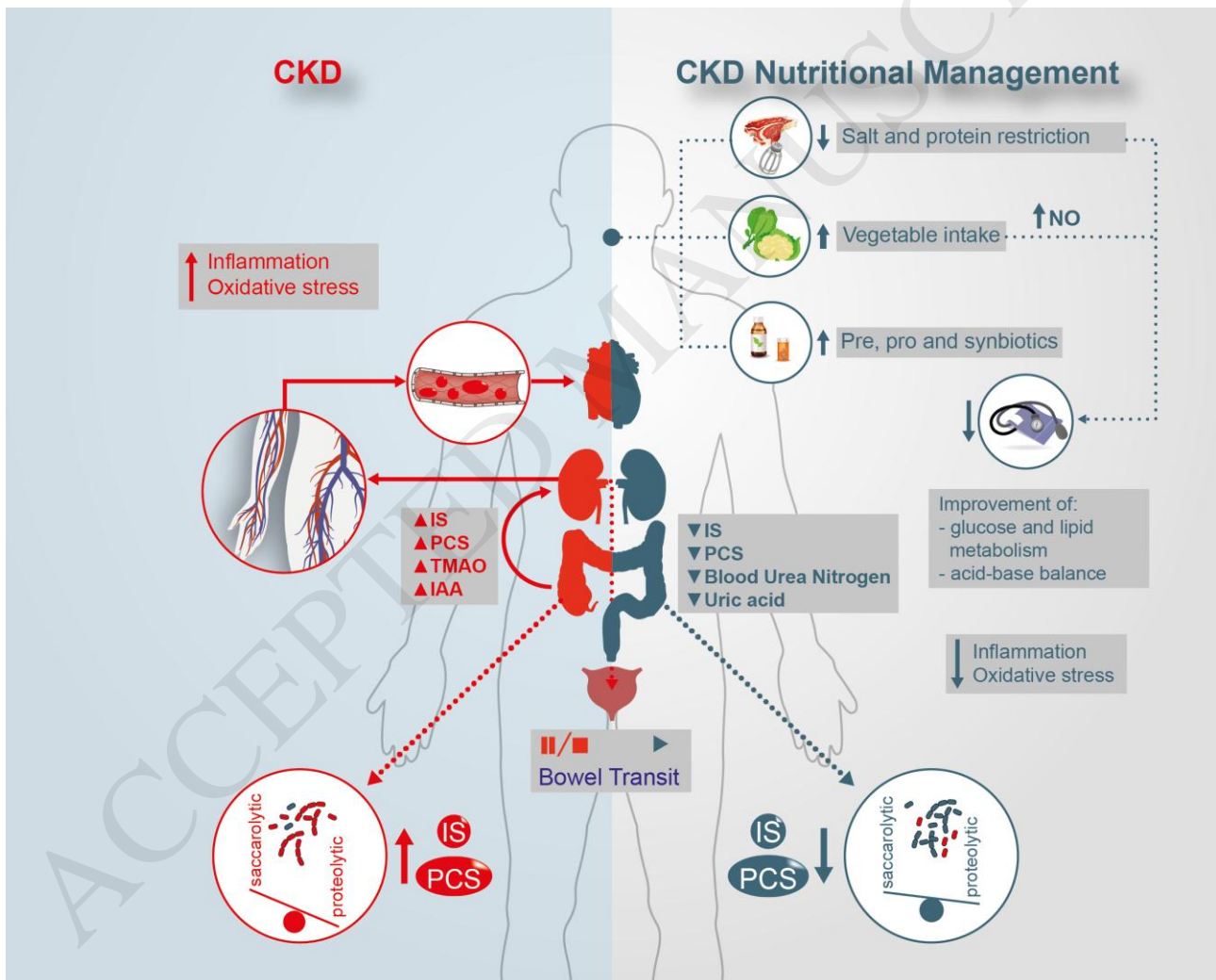


Table 1. Main uremic toxins produced by gut microbiota and their production/excretion pathways

Abbreviations: p-cresyl sulfate (PCS), indoxyl sulfate (IS), trimethylamine-N-Oxide (TMAO), indole-3 Acetic Acid (IAA), organic anion transporter (OAT), trimethylamine (TMA), flavin monooxygenase (FMO), organic cation transporter 2 (OCT2), ATP-binding cassette (ABC), tryptophan 2,3-dioxygenase (TDO), indoleamine 2,3-dioxygenase (IDO)

Uremic Toxin	Substrate	Synthesis and Excretion
PCS [5,27,30]	Dietary proteins (Tyrosine and Phenylalanine)	<i>Colon bacteria</i> : chemical deamination, transamination, and decarboxylation of tyrosine and phenylalanine → p-cresol <i>Liver and enterocytes</i> : sulfation of p-cresol → PCS PCS is transported through OAT1 and OAT3 by proximal renal tubular cells and secreted in the urine
IS [5,27]	Dietary proteins (Tryptophan)	<i>Colon bacteria</i> : tryptophan → indole by microbial tryptophanase <i>Liver</i> : oxidation and sulfation of indole → IS IS is transported through OAT1 and OAT3 by proximal renal tubular cells and secreted in the urine
IAA [51,52, 131]	Dietary proteins (Tryptophan)	<i>Colon bacteria</i> : tryptophan → indole by microbial tryptophanase <i>Gut and tissue</i> : Indole by tryptamine → IAA IAA is transported through OAT by proximal renal tubular cells and secreted in the urine. ³
TMAO [66,68, 132]	Dietary choline, phosphatidyl choline and L-carnitine	<i>Colon bacteria</i> : Phosphatidylcholine and choline by choline trimethylamine-lyase → TMA L-carnitine by gut microbial lyase → TMA <i>Liver</i> : oxidation of TMA by FMO3 → TMAO Few studies about TMAO excretion, that seems to undergo uptake through OCT2 and ABC transporters by proximal renal tubular cells and urinary excretion

Table 2. Main experimental and clinical studies investigating the effects of gut microbiota metabolites in CV complications

Experimental studies		
Model	Observed effect	reference
<i>In vitro</i> (HUVEC incubated with PCS and IS)	Decreased endothelial proliferation and wound repair by PCS and IS	Dou et al. Kidney Int. 2004. doi:10.1111/j.1523-1755.2004.00399.x
<i>In vitro</i> (HUVEC incubated with IS)	Enhanced ROS production, increased NAD(P)H oxidase activity and decreased glutathione levels in endothelial cells by IS	Dou et al. J Thromb Haemost. 2007. doi: 10.1111/j.1538-7836.2007.02540.x
<i>Ex vivo</i> (whole blood cells)	Pro-inflammatory effect (oxidative burst activity induction) on unstimulated leucocytes by PCS	Schepers et al. Nephrol Dial Transplant. 2007. doi: 10.1093/ndt/gfl584
<i>In vivo</i> (Dahl salt-resistant normotensive rats and Dahl salt-sensitive hypertensive rats administered with different salt intake and IS)	Induction of aortic calcification with expression of osteoblast-specific proteins and aortic wall thickening by IS	Adijiang et al. Nephrol Dial Transplant. 2008. doi: 10.1093/ndt/gfm861
<i>In vitro</i> (HUVEC pre-treated with IS and activated with TNF- α) <i>In vivo</i> (nephrectomized chronic kidney disease model mice)	Enhanced leukocyte-endothelial interactions through up-regulation of E-selectin by IS	Ito et al. J Biol Chem. 2010. doi: 10.1074/jbc.M110.166686
<i>In vivo</i> (Atherosclerosis-prone mice C57BL/6J.ApoE $^{-/-}$)	Upregulation of multiple macrophage scavenger receptors linked to atherosclerosis, and atherosclerosis promotion by supplementation with choline or TMAO in mice. Augmented endogenous macrophage foam cell formation induced by dietary choline. Inhibition of dietary-choline-enhanced atherosclerosis by suppression of intestinal microbiota. Genetic variations in liver enzymes (FMOs) leading to TMAO production associated with atherosclerosis in hyperlipidaemic mice.	Wang et al. Nature. 2011. doi: 10.1038/nature09922
<i>In vitro</i> (HUVEC incubated with IS)	Induced production of ROS in HUVEC by IS. Pretreatment with antioxidants ameliorated IS-induced inhibition of proliferation and nitric oxide production and senescence inhibition of HUVEC	Yu M et al. Clin J Am Soc Nephrol. 2011. doi: 10.2215/CJN.05340610
<i>In vitro</i> (HUVEC incubated with different protein-bound uremic toxins)	Induction of endothelial ROS production by IS and 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid (CMPF)	Itoh et al. Anal Bioanal Chem. 2012. doi:10.1007/s00216-012-5929-3
<i>In vivo</i> (C57BL/6J ApoE $^{-/-}$ mice given L-carnitine)	Downstream events following L-carnitine ingestion in mice: increased	Koeth et al. Nat Med. 2013. doi:

	TMAO levels, accelerated atherosclerosis and reduction of reverse cholesterol transport	10.1038/nm.3145
<i>In vitro</i> (Human proximal tubular cells HK-2 stimulated with IS)	Upregulation of angiotensin expression in HK-2 by IS (mediated by CREB, NF- κ B, and NOX4)	Shimizu et al. Am J Physiol Cell Physiol. 2013. doi: 10.1152/ajpcell.00236.2012
<i>In vivo</i> (Dahl salt-resistant normotensive rats and Dahl salt-sensitive hypertensive rats administered with different salt intake and IS)	Worsening of cardiac fibrosis and cardiomyocyte hypertrophy with enhanced oxidative stress and reduced anti-oxidative defense in hypertensive rats by IS	Yisireyili et al. Life Sci. 2013. doi: 10.1016/j.lfs.2013.05.008
<i>In vitro</i> (cultured human endothelial cells)	Endothelial inflammation, oxidative stress and inflammatory AhR/p38MAPK/NF- κ B pathway activation by IAA	Dou et al. J Am Soc Nephrol. 2015. doi: 10.1681/ASN.2013121283
<i>In vivo</i> (LDLR(-/-) mice) <i>In vivo</i> (primary human aortic endothelial cells and vascular smooth muscle cells)	Choline and TMAO-induced inflammatory gene expression in aortas of mice compared with controls and in human endothelial and vascular smooth muscle cells. TMAO-induced recruitment of activated leukocytes to endothelial cells.	Seldin et al. J Am Heart Assoc. 2016. doi: 10.1161/JAHA.115.002767
<i>Ex vivo</i> (human platelets) <i>In vivo</i> (carotid artery thrombosis mouse model and germ-free C57BL/6J mice)	Enhanced sub-maximal stimulus-dependent platelet activation by TMAO. Dietary choline, TMAO and specific gut microbial taxa enhance platelet responsiveness and <i>in vivo</i> thrombosis potential. Thrombosis potential transmissible by microbiota transplantation <i>in vivo</i> .	Zhu et al. Cell. 2016. doi: 10.1016/j.cell.2016.02.011

Human studies

Study population	Main results	Reference
Data collected from two cohorts (n=1876) involving stable non-symptomatic subjects undergoing elective cardiac evaluations at a tertiary care center (GeneBank and Biobank)	Elevated levels of choline, TMAO and betaine dose-dependent associated with the presence of CVD and multiple individual CVD phenotypes including peripheral artery disease, coronary artery disease, and history of myocardial infarction	Wang et al. Nature. 2011. doi: 10.1038/nature09922
Prospective observational study in 40 CKD patients, 24 weeks duration	AST-120 treatment for 24 weeks resulted in a significant increase in FMD with a decrease in IS and oxidized/reduced glutathione ratio.	Yu M et al. Clin J Am Soc Nephrol. 2011. doi: 10.2215/CJN.05340610
70 pre-dialysis patients, 36-month follow-up	IS significantly associated with CV and dialysis event.	Lin et al. Arch Med Res. 2012. doi: 10.1016/j.arcmed.2012.08.002
GeneBank cohort of subjects	Plasma L-carnitine levels predicted	Koeth et al. Nat Med.

(n=2595 included in the study) undergoing elective cardiac evaluation	increased risks for both prevalent CVD and incident major adverse cardiac events (myocardial infarction, stroke or death), but only among subjects with concurrently high TMAO levels.	2013. doi: 10.1038/nm.3145
Prospective study in 120 CKD patients, follow-up ≈ 3 years	Mortality and CV events associated with IAA serum levels in CKD patients. Serum IAA significant predictor of mortality and CV events by multivariate Cox regression analysis (after adjustments). IAA levels positively correlated with markers of inflammation and oxidative stress.	Dou et al. J Am Soc Nephrol. 2015. doi: 10.1681/ASN.2013121283
Meta-analysis of 10 prospective and 1 cross-sectional study Total CKD (> stage 3) patients included = 1572	PCS associated with an increased risk of CV events in CKD patients. Elevated levels of PCS and IS associated with increased mortality in CKD patients.	Lin et al. PLoS One. 2015. doi:10.1371/journal.pone.0132589
Association study on 394 participants of a US national prospective cohort study of incident dialysis patients	PCS levels associated with CV mortality risk; PCS and phenylacetylglutamine levels associated with first CV event. Patients in the highest quintile of the combined solute index had a 96% greater risk of CV mortality and 62% greater risk of first CV event compared with patients in the lowest quintile.	Shafi et al. PLoS One. 2015. doi:10.1371/journal.pone.0126048
Cohort of sequential stable subjects (n = 4,007) presenting to a cardiology clinic for elective diagnostic cardiac evaluations; 3-year longitudinal follow-up	Plasma TMAO levels independently predicted incident (3 years) thrombosis (heart attack, stroke) risk.	Zhu et al. Cell. 2016. doi: 10.1016/j.cell.2016.02.011
Meta-analysis of 19 prospective studies (n=19256 patients, including 3315 incident cases)	Higher circulating levels of TMAO and precursors associated with an increased risk of major adverse cardiovascular events, regardless of conventional risk factors.	Heianza et al. J Am Heart Assoc. 2017. doi: 10.1161/JAHA.116.004947

Abbreviations: chronic kidney disease (CKD), cardiovascular disease (CVD), flow-mediated dilation (FMD), human umbilical vein endothelial cells (HUVEC), indole-3 Acetic Acid (IAA), indoxyl sulfate (IS), p-cresyl sulfate (PCS), reactive oxygen species (ROS), trimethylamine-N-Oxide (TMAO), tumor-necrosis factor- α (TNF- α)