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A Renal Clinician's Guide to the Gut Microbiota

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Abstract

It is increasingly recognised that the gut microbiota plays a role in the progression of chronic diseases, and that diet may confer health benefits by altering the gut microbiota composition. This is of particular relevance for chronic kidney disease (CKD), as the gut is a source of uremic retention solutes which accumulate as a result of impaired kidney function and can exert nephrotoxic and other harmful effects. Kidney dysfunction is also associated with changes in the composition of the gut microbiota and the gastrointestinal tract. Diet modulates the gut microbiota and there is much interest in the use of pre-, pro- and synbiotics as dietary therapies in CKD, as well as dietary patterns that beneficially alter the microbiota. This review provides an overview of the gut microbiota and its measurement, its relevance in the context of CKD and the current state of knowledge regarding dietary manipulation of the microbiota.

Introduction

The role of the gut microbiota and its implications for health and disease have evolved rapidly over the last decade, with studies suggesting that the gut microbiota (the community of bacteria that reside in the gastrointestinal tract) has an integral role in modulating the risk of several chronic diseases, including kidney disease. Indeed, alterations to the gut microbiota have been repeatedly linked to diseases such as inflammatory bowel disease[1], cardiovascular disease[2], chronic kidney disease (CKD)[3], obesity[4,5], type 2 diabetes[6] and cancer.[7] The gut microbiota is variable with diet and antibiotic use significantly impacting its composition and diversity[8]. Increasingly, studies have demonstrated the significant role

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diet plays in shaping the gut microbiota with proof-of-concept studies demonstrating it is amenable to therapeutic intervention.[9]

Scientific research and general public interest in the use of gut targeted therapies, such as pro- , pre- and synbiotics, as an adjunct to traditional medical therapy to treat or manage chronic diseases has grown substantially. It is estimated that two out of every three adults use dietary herbal, vitamin and/or mineral supplements to help manage their chronic conditions.[10] This trend is reflected in supplements targeting gut health with sales in probiotics predicted to reach in excess of $64 billion by 2023.[11] Patients with CKD have highlighted their desire for a preventative approach to managing their health and wellbeing[12] and seek out expert dietitians for individualised nutrition advice.[13] It is likely that these desires align with the therapeutic potential of gut health supplementation. As such, dietitians are in a key position to advise patients with CKD about the evidence and limitations of supplements aimed to improve gut health.[14]

The aim of this article is to provide an overview of the gut microbiota and its measurement, describe the link between gut microbiota and clinical outcomes in CKD, and outline potential dietary strategies to manipulate the gut microbiota.

Meet the Microbiota

The terms ‘microbiome’ and ‘microbiota’ are often used synonymously, however they have specific meanings (Table 1). The term ‘microbiota’ refers to a collection of microorganisms that are found in a particular sample or location, whilst the ‘microbiome’ refers to the collective genetic material of a microbial community. Approximately 500-1000 bacterial species are present in the community that is the gut microbiota[15], with many bacteria sharing similar genes that can perform similar functions[16], known as genetic redundancy. Many bacterial genes encode for proteins that possess functions such as fermentation of dietary fibre or the production of Vitamin K, that the human genetic component is not capable of. Here we use the term microbiota to refer to bacteria as most studies that refer to the microbiota, particularly the gut microbiota, are focused on the bacterial organisms that inhabit that ecosystem. However, it is important to note that a number of other microorganisms inhabit the gut including Archaea (many of which are methane producers), viruses (the virome), fungi (the mycobiome) and bacteriophages (the phageome).[17]

Measuring Microbes

Up until the 1990s, the primary method for assessment of the microbiota was the use of culture-based techniques.[18] As this involved the use of selective growth media, permitting the growth of particular types of bacteria whilst restricting the growth of others, it enabled enumeration of selectively grown bacteria. For example, to ascertain the relative abundance of lactobacilli between two samples, a dilution would be plated out onto the agar plate and allowed to incubate. Following incubation, the number of bacterial colonies that were visible on the plate would be counted and from this a concentration of colony forming units could be calculated. This technique has the benefit of being relatively cheap and for many years was considered the gold standard for microbial identification.[18] However, less than 30% of

gut bacteria are culturable using current culture techniques.[19] Furthermore, this approach does not typically allow the differentiation between bacterial species or strains. Over the past two decades, there have been notable advances in the development of culture-independent techniques, that has greatly broadened our understanding of the microbiota. These include DNA microarrays, targeted PCR (qPCR), denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP) and fluorescence in situ hybridization (FISH), which have been described in detail elsewhere.[18] Whilst useful for advancing our understanding of the microbiota, these techniques have largely been superseded over the past decade by next generation sequencing (NGS) methodologies. The relevance and application of sequencing methodologies, as it applies to research and practice in CKD will be detailed below.

Sequencing

The primary driver behind the advancing knowledge base and interest in the microbiome (as well as genomics in general) is the decrease in the cost of sequencing, largely due to the development of NGS technologies.[18] Rather than trying to culture specific bacterial organisms, these approaches identify the genetic material from the bacterial samples, and use this to determine bacterial identity. This has the benefit of identifying both non-culturable and culturable bacteria. There are two main NGS methods that are commonly used for gut microbiota work: 16S rRNA sequencing and metagenomics (often referred to as shotgun sequencing).[20] Metagenomics involves sequencing all of the bacterial DNA within a sample, whilst 16S rRNA sequencing only sequences a portion of the bacterial 16S rRNA gene.[21]

16S Sequencing

All bacteria have a portion of their genome which codes for the highly conserved 16S rRNA gene, coding for 16S ribosomal RNA, which forms part of the ribosome.[20] This 16S rRNA gene contains nine highly variable regions, named V1 to V9, which can be used to differentiate between bacterial species.[22] Typically, a particular region of the 16S gene will be used, such as the V3-V5 region. Sequencing of these 16S rRNA gene regions can be compared with databases of previously classified 16S sequences to allow for identification of the bacteria that sequence originated from.[20] Commonly used databases for 16S sequences classification are Greengenes[23], SILVA[24] and the Ribosomal Database Project (RDP).[25] The level of resolution provided by this approach is limited, with not all bacteria being able to be identified to a species or genus level. If you are interested in performing 16S rRNA sequencing, Claesson et al.[21] provides an excellent starting overview. 16S sequencing provides greater insights into the bacterial community than could be achieved by previous methods, however there are limits in terms of the depth and resolution that can be achieved.[20]

Shotgun sequencing (aka metagenomics)

Metagenomic shotgun sequencing provides the most informative view of the microbiome, however generates vast volumes of data, is quite computationally intensive and requires specialised bioinformatics skills.[21] Metagenomics involves the sequencing of all microbial DNA within a sample. This is achieved by shearing bacterial DNA into fragments, which are

subsequently sequenced simultaneously (known as massive parallel sequencing).[26] These sequences then need to be aligned into a continuous sequence to represent the bacterial genome, a process known as metagenome assembly.[20] The process of metagenome assembly requires significant compute power and requires access to a high performance computing cluster.[26] Metagenomics has the advantage of characterising bacteria to the strain level, which 16S rRNA sequencing cannot, however is significantly costlier and requires specialised skill sets and access to high-powered computing resources.

Non-Genetic Techniques for Microbiota Analysis

Whilst the use of culture-based and sequencing techniques detailed above may answer the question of form, i.e. “who’s there?”, there are a number of techniques that seek to answer the question of function, i.e. “what are they doing?”. These are based around the central dogma of biology, namely that genes are transcribed into messenger RNA (mRNA) which are translated into proteins, which can have functional impact for the organism. Critically, the commonly used sequencing based techniques for identifying the gut microbiota discussed above do not give rise to information about function, nor discriminate between alive and dead bacterial cells.

Metatranscriptomics and Metaproteomics

Metatranscriptomics involves the sequencing of total microbial mRNA whilst metaproteomics involves identifying all microbial proteins in a sample.[27] A bacterium may possess a particular gene, however if that gene is not transcribed into mRNA and subsequently translated into a protein, there is no opportunity for a functional effect to occur. For example, data from metagenomic sequencing indicated that the microbiome of participants contained genes for tryptophan biosynthesis, however the mRNA transcripts of these genes were consistently underexpressed.[28] It was suggested that, due to the high bioavailability of tryptophan in the host diet, it was not favourable for the bacteria to expend the energy synthesising the proteins required to make tryptophan, when it was freely available in the environment. Whilst metagenomic sequencing provided an indication of the genetic potential of the microbiome, the use of metatranscriptomics provided a greater insight into the function of the microbiota.

Metabolites and Metabolomics

One of the primary ways in which the microbiota has an effect on the host is by the production of microbial metabolites and the measurement of these metabolites is one way to assess the effect of an intervention on the microbiota.[29] The metabolome refers to the complete set of metabolites present in a particular sample. Commonly assessed microbial metabolites that have relevance for CKD populations include uremic retention solutes, detailed below, and short-chain fatty acids (SCFAs).[30] These metabolites may be measured in faeces, blood or urine. The measurement of specific metabolites of microbial origin provide an insight into the effects of diets and drugs on the microbiota.

Sampling and Processing Effects

Most studies in humans utilise stool samples as a proxy that is indicative of the distal colonic luminal microbiota. The mucosal microbiota, that is the bacteria that are closely associated with the intestinal mucus layers, is significantly different from the fecal microbiota.[31–33] The mucosal microbiota has close interactions with intestinal epithelial cells, as well as those of the immune system, and may have relevance for the integrity of the gastrointestinal barrier. In experimental animal models of CKD, it has been shown that gastrointestinal permeability is increased with CKD[34–36], and markers of intestinal permeability are inversely correlated with decreasing estimated glomerular filtration rate (eGFR) in CKD patients.[37,38] Of practical relevance for patients is that an endoscopy is required for obtaining mucosa-associated microbes.[33] Furthermore, the bowel preparation required for a colonoscopy changes the composition of the stool microbiota[39] and it was recently shown to also alter the composition of the mucosal microbiota.[40] To decrease patient burden the majority of human microbiota studies utilise stool samples, however it is important to understand the potential limitations with this approach.

Another important consideration with regards to stool sampling is that the bacterial composition of the stool is not uniform, and the area of sampling may affect results. Sampling from the exterior of the stool sample resulted in significantly lower abundance of *Bifidobacteria*, compared with an interior sample, a fact ascribed to the anaerobic nature of *Bifidobacteria*,[41] Another study, which obtained samples from the same stool 1cm apart, observed differences in the microbial composition between the samples, particularly for low abundance taxa.[42] Homogenisation of the entire sample, as opposed to taking a subsample from a section of the stool, has been identified as a technique to limit this variability.[41] Whilst this approach has been recommended[41], it may be impractical to implement this in the field setting.[43]

Following sample collection, the subsequent shipping and storage conditions may also impact the microbiome. Whilst there is some inconsistency with regards to whether freezing alters the composition of the gut microbiome[44–47] it has been recommended that, where possible, DNA extraction be conducted on freshly collected samples.[48] Understandably this is not always possible, in which case samples should be stored at −80°C as soon as possible.[49] When immediate freezing is not possible, the use of a commercial stabilising kit such as the OMNIgene gut tube is recommended.[50] If SCFA analysis is to be undertaken, it is recommended that samples be frozen at −80°C immediately, as these compounds are highly volatile.[51]

Finally, the bacterial DNA extraction method may also impact the microbiota. Costea et al.[52] assessed 21 DNA extraction protocols utilized in research and established a benchmark method for DNA extraction based on DNA quantity, quality and community diversity. This benchmark DNA extraction protocol, standards of practice for sample collection methods, and visual aids can be found in the International Human Microbiome Standards (IHMS) website (www.microbiome-standards.org/).

The Gut Microbiota and Renal Disease

Kidney dysfunction induces changes in the gastrointestinal tract

There are changes in the gastrointestinal tract as kidney function declines. Specifically, there is an effect of urea which may directly damage the epithelial cells of the gastrointestinal tract. Experimentally, Vaziri et al.[53] demonstrated that, in a cell line of human enterocytes, a concentration of urea similar to that observed in patients with end-stage kidney disease (ESKD), leads to a decrease in several tight junction proteins. In the same study, the presence of urease, a bacterial enzyme that metabolizes urea into ammonia, was associated with an even greater reduction in tight junction proteins. Tight junction proteins play a pivotal role in maintaining the integrity of the gastrointestinal epithelial barrier and a decrease in these tight junction proteins leads to increased intestinal permeability, permitting the translocation of bacteria or bacterial fragments into the systemic circulation.

While it is generally impractical to assess changes in gastrointestinal epithelial tight junction proteins in individuals with CKD, bacterial translocation can be assessed through the measurement of circulating levels of endotoxin. Indeed, several studies have reported higher endotoxin levels in patients with advancing grades of CKD. McIntyre at al.[37] observed a negative association between eGFR and level of circulating endotoxin, with the highest level in those undergoing dialysis. Recently, Shah et al.[54] conducted a pilot study comparing the blood microbiome of patients with normal and reduced kidney function. Whilst patients with CKD had a lower bacterial richness and a higher relative abundance of several bacteria, primarily in the Enterobacteriaceae and Pseudomonadaceae families, it is interesting to note that there was no difference in microbial DNA concentration in the blood between groups.[54] Overall, these data suggest that kidney dysfunction affects gastrointestinal tract integrity and increases intestinal permeability.

Changes in the gut microbiota with CKD

Concurrent with changes in gastrointestinal tract permeability, there are changes in the gut microbiota with CKD. Animal models of CKD have been shown to have a different microbial composition compared to littermates with normal kidney function. For example, Vaziri et al.[3] reported that colonic contents from 5/6 nephrectomy rats had a reduced relative bacterial richness compared to sham-operated rats. Furthermore, the relative abundance of Betaproteobacteria class was enriched, while the Lactobacillaceae and Prevotellaceae families were reduced in CKD rats. Similarly, in fecal pellets from 5/6 nephrectomy mice, Nishiyama et al.[55] showed that at the genus level, there was an increase in the relative abundance of *Allobaculum, Bifidobacterium*, and *Turicibacter*, while there was a decrease in *Lactobacillus, Oscillospira*, unclassified Ruminococcaceae, and unclassified Rikenellaceae, compared to sham-operated mice.

Assessing the gut microbiota of colonic contents or fecal pellets is a closer indication to the luminal distal microbiota as mentioned above. However, some researchers have assessed the effects of kidney dysfunction in the cecal digesta in animal studies, which is a more representative indication of the proximal large intestine microbiota. Lau et al.[56] observed that 5/6 nephrectomy rats had lower bacterial diversity and a higher relative abundance of

the phylum Bacteroidetes and fewer Firmicutes, compared to rats with normal kidney function. In adenine-induced CKD mice, Yang et al.[57] found that there were 17 genera that were different between normal and CKD mice. Particularly, *Dorea, Coprobacillus, Clostridium, Ruminococcus, Lactobacillus*, unclassified Erysipelotrichaceae, *Staphylococcus, Allobaculum*, and *Blautia* were enriched in the CKD mice cecal digesta. While these studies are informative, they may not translate into human studies due to the difficulty and invasiveness that would constitute obtaining proximal colon samples.

Experimental studies have provided an indication that the gut microbiota changes with kidney dysfunction. However, it is important to note that there are key differences in the gastrointestinal tract between mammals and some animal traits do not translate into human studies. These differences include the predominant site of bacterial fermentation, where rodents are cecal fermenters, while humans are colonic fermenters.[58] In addition, rodents are a coprophagic species, a trait which alters the microbiome.[59] In terms of the gut microbiota composition, at the phyla level, Firmicutes and Bacteroidetes are the most abundant in both humans and murines. However, up to 85% of the bacteria genera in mice are not present in humans and, therefore, comparisons of the gut microbiota of murines and humans should be made with caution.[60]

In humans, changes in the gut microbiota may start early in the development of CKD. Vaziri et al.[3] reported that individuals with ESKD undergoing hemodialysis treatment had a higher relative abundance of Actinobacteria, Firmicutes, and Gammaproteobacteria, while individuals without CKD had a higher relative abundance of Lactobacillaceae, Sutterellaceae, and Bacteroidaceae families. Crespo-Salgado et al.[61] compared the fecal microbiota of pediatric patients undergoing peritoneal dialysis, hemodialysis, post-kidney transplant and healthy controls. Peritoneal dialysis and post-transplant patients had lower bacterial species richness compared with patients on hemodialysis and healthy controls. At the phyla level, patients undergoing peritoneal dialysis had a lower relative abundance of Firmicutes and Actinobacteria, while pediatric patients on hemodialysis had a higher relative abundance of Bacteroidetes. At the family level, peritoneal dialysis patients had an expansion of Enterobacteriaceae and a contraction of Bifidobacteriaceae. In a targeted quantitative real-time PCR analysis, Wang et al.[62] observed that adult patients undergoing peritoneal dialysis had decreased relative abundances of *Bifidobacterium* and *Lactobacillus spp.* including *B. catenulatum, B. longum, B. bifidum, L. plantarum*, and *L. paracasei.* Therefore, somewhat consistently it has been observed that in patients with CKD, the relative abundance of symbiotic bacteria is reduced, while some potential pathobionts are increased. However, it is still unclear if shifting the gut microbiota composition or increasing the relative abundance of symbiont bacteria improves outcomes in CKD patients.

While there are changes in the gastrointestinal microbiota with kidney dysfunction, the results are not always consistent between studies. One of the limitations is the diversity in protocols utilized, including different sample processing methods, sequencing techniques and analysis platforms. In addition to knowing the composition of the gut microbiota, the functional capacity differences may be of more clinical relevance. Wong et al.[63] observed that in patients undergoing hemodialysis, there was an expansion in the bacterial families that possess urease, uricase, and indole and p-cresol forming enzymes and a decrease in

bacterial families with butyrate-forming capacity. Similarly, Jiang et al.[64] reported that there is a decrease in the butyrate-producing bacteria *Roseburia spp.* and *Faecalibacterium prausnitzii* with even a mild reduction in kidney function compared to healthy individuals, but it is important to note that they used a targeted qPCR approach which only detected these bacterial species. By understanding the bacterial capacity for producing particular metabolites, we can gain further insight into the relevance of the microbiota to CKD.

Uremic retention solutes

As kidney function declines, there is an increased accumulation of molecules normally cleared by the kidneys, collectively called uremic retention solutes. These uremic retention solutes include metabolites of microbial metabolism. The European Uremic Toxin Work Group has published a variety of articles related to uremic retention solutes, with helpful information including reference values.[65] The most widely studied uremic retention solutes derived of microbial metabolism are indoxyl sulfate, p-cresyl sulfate, and trimethylamine-N oxide (TMAO) (Figure 1). These uremic retention solutes have been associated with an increased risk of kidney disease progression[66,67], cardiovascular disease[68,69], mineral and bone disorder[70], and even mortality.[71,72]

Indoxyl Sulfate

Indoxyl sulfate is derived from the microbial metabolism of dietary tryptophan to indole, through the bacterial enzyme tryptophanase.[73] Tryptophanase activity is facilitated at higher pH and, therefore, occurs mostly in the distal colon.[74] However, patients with CKD often consume a low-fiber diet due to dietary restrictions to limit phosphorus and potassium intake[75,76], leading to a reduction in colonic SCFA production. This reduction in SCFA production may lead to an increase in luminal pH in the proximal colon, facilitating tryptophanase activity across the length of the colon. The indole cleaved from tryptophan is absorbed into the portal circulation. In the liver, the indole is oxidized to indoxyl and sulfated leading to the formation of indoxyl sulfate.[77] Ninety to 98% of indoxyl sulfate is bound to plasma proteins, such as albumin, and is excreted in urine by tubular secretion through organic anion transporters.[78]

Plasma indoxyl sulfate increases as kidney function declines, and those receiving dialysis treatment have the highest serum concentration.[79] The European Uremic Toxin Work Group reported that comparing the average concentration of indoxyl sulfate in uremia to normal concentrations, CKD patients have ~40-fold higher total indoxyl sulfate.[65] Elevated indoxyl sulfate concentrations in dialysis patients are in part due to the fact that traditional dialysis treatment does not replicate the kidney's high efficiency of tubular secretion.[78] Some of the deleterious effects of indoxyl sulfate include increased oxidative stress through the activation of NADPH-oxidases, enhanced inflammatory state through the activation of NF-kB via STAT3, activation of the renin-angiotensin-aldosterone system (RAAS), enhanced aortic calcification, and mineral and bone disorder, all of these reviewed by Leong and Sirich.[78]

Observational and controlled trials have targeted the production, absorption, and removal of indoles and indoxyl sulfate. Some of the mechanisms to decrease circulating indoxyl sulfate include limiting the production of indole by very-low[80] and low-protein diets[81], therapies

targeting the gut microbiota (i.e., pro-, pre-, and synbiotics[82]), reduction of indole absorption through adsorptive therapies (i.e., AST-120[83,84] and sevelamer[85–88]) and enhanced removal via hemodiafiltration.[89,90] Recently, the infusion of an albumin binding competitor, ibuprofen, during dialysis led to a reduction in serum levels of indoxyl sulfate.[91] Overall however, the evidence for any single approach is uncertain, due to inconclusive and/or small clinical trials to date.

P-cresyl Sulfate

P-cresol is derived from the fermentation of tyrosine through microbial metabolism.[92] In the liver, p-cresol is sulfated by aryl sulfotransferase forming p-cresyl sulfate.[93] Similarly to indoxyl sulfate, in circulation 90-98% of p-cresyl sulfate is protein-bound and is excreted in urine by tubular secretion through the organic anion transporters[94], albeit to a lower extent than indoxyl sulfate.[95] Serum p-cresyl sulfate concentrations increase as kidney function declines, with the highest concentrations being observed in ESKD and dialysis patients.[79,95] The European Uremic Toxin Work Group reported that comparing the average concentration of p-cresyl sulfate in uremia to normal concentrations, CKD patients have ~21-fold higher total p-cresyl sulfate.[65] P-cresyl sulfate has been associated with poor outcomes in experimental, observational, and clinical studies. At the renal level, p-cresyl sulfate causes renal tubular damage through activation of NADPH-oxidases.[96] Additionally, p-cresyl sulfate has been associated with increased inflammation[97], reduced antioxidant capacity[97], activation of RAAS, increased insulin resistance, cardiovascular risk, all-cause and cardiovascular mortalities in patients with CKD.[66,93,98,99]

Several observational and clinical trials have targeted indoxyl and p-cresyl sulfate with approaches such as low-protein diets[100], supplementation of dietary fiber (i.e., resistant starch[101,102], arabinoxylan oligosaccharides[103]), synbiotics[9,82] and hemodiafiltration. Recently, the infusion of a binding competitor, ibuprofen, during dialysis led to a reduction in serum levels of indoxyl sulfate and p-cresyl sulfate.[91] However, results have been inconsistent and comprehensive studies are still needed to assess the effect on outcomes with the reduction on p-cresyl sulfate.

Trimethylamine-N oxide (TMAO)

In the colon, dietary choline, carnitine, and γ-butyrobetaine are cleaved to form trimethylamine, which is absorbed intestinally and oxidized in the liver to trimethylamine-N-oxide (TMAO).[82–8485] Contrary to indoxyl sulfate and p-cresyl sulfate, TMAO is not protein-bound, and is cleared through tubular secretion or hemodialysis.[104] The interest in TMAO increased substantially after Wang et al.[2] reported that metabolites derived from microbial metabolism of phosphatidylcholine were associated with increased risk of major adverse cardiovascular mortality. Further experimental and clinical studies revealed that other molecules with a trimethylamine moiety, such as carnitine[105] and γ-butyrobetaine[106] acted in a similar fashion. Mechanistically, TMAO promotes atherosclerosis by enhancing macrophage cholesterol accumulation.[107] Tang et al.[67] demonstrated that TMAO also caused progressive kidney fibrosis experimentally and clinically was associated with progression of CKD. TMAO increases as kidney function declines, with concentrations reported up to ~40-fold higher in hemodialysis patients before dialysis compared with healthy controls.[108]

Diets that are predominantly animal-based have been shown to increase levels of TMAO, in comparison to predominantly plant-based diets.[109] Cho et al.[110] compared the effects of a single meal of fish (naturally high in TMAO) with foods containing dietary precursors to TMAO, eggs (choline) and beef (carnitine). They showed that circulating TMAO was significantly higher following the fish meal compared to either eggs or beef. Despite reports of interventions to reduce TMAO levels in other clinical populations, there have been limited interventions to reduce TMAO levels in patients with CKD. Recently, Borges et al.[111] showed no effect on TMAO concentrations following a 4-week supplementation with probiotics. Despite the lack of evidence, several researchers have hypothesized that the reduction of meat consumption, plant-based diets, low-protein diets, and interventions targeting the gut microbiota composition may lower TMAO levels in CKD and improve outcomes, particularly decreasing cardiovascular risk.[112,113]

Short-chain fatty acids (SCFA)

Whilst uremic retention solutes are microbially produced metabolites that may harm the host, there are some end products of microbial metabolism that have a beneficial effect for the host. SCFAs are mostly derived from the bacterial fermentation of non-digestible carbohydrates that reach the colon. SCFAs have a carbon chain length of less than six, with acetate (C2), propionate (C3), and butyrate (C4) being the most abundant, produced in the proportion of 60:20:20 of acetate, propionate, and butyrate, respectively.[114] In comparison to uremic retention solutes, SCFA are desirable as they have several physiological benefits, including enhancing gut barrier function[115], increasing production of intestinal hormones (i.e. glucagon-like peptide 1[116]), improving metabolic health (i.e. glucose and lipid metabolism[117]), enhancing mineral absorption (i.e. calcium, magnesium, and iron[118]), among other benefits.[114]

Implications for Practice

Nutrition supplementation with prebiotics, probiotics and synbiotics have emerged over the last decade as potential strategies to manipulate the microbiota and restore gut health. Prebiotics, substrates that are selectively utilised by the host microorganism to confer a health benefit[119], alter the carbohydrate-to-protein ratio in the gut resulting in increased production of SCFAs, decreased colonic pH, reduced colonic transit time and the modulation of the colonic microbiota.[120] Prebiotics occur naturally in many foods, such as onion, garlic and bananas[121], however they make up only a small percentage of the Western diet. More commonly, prebiotics, such as inulin and fructo-oligosaccharides, are added to foods, such as yoghurt, breads and cereals, to form functional foods.[121] Probiotics, live beneficial bacteria, limit the intestinal concentration of potentially pathogenic microorganisms through colonisation resistance or competitive exclusion.[122] The most common commercially available probiotic strains belong to the following genera: *Lactobacillus, Streptococcus* and *Bifidobacterium*.[121] In other chronic diseases, the results from probiotic interventions have been strain specific[123], with only certain strains and doses achieving meaningful clinical outcomes. Therefore, commercial probiotic preparations tend to include multiple bacterial strains. Synbiotics, the co-administration of pre- and probiotics, may be effective in re-establishing normal gut microbiome in other chronic diseases.[124] The ability to select

specific prebiotics that promote the growth of bacterial genera is a key benefit of synbiotic therapy.[125]

The benefit of pre-, pro- and synbiotic supplementation has been well established in several conditions[126], however, as detailed above, have uncertain effect in CKD. Trials in this area have been hampered by study design limitations and produced conflicting results.[82] Potential benefits of supplementation in the CKD population include reductions in uremic retention solutes[127], improvements in glycaemic response[128] and decreased triglycerides.[129] In addition, there has been an associated high level of patient compliance and lack of reported side-effects.[9] Whilst these results are promising, there remains limited evidence to support the routine recommendation of pre-, pro- or synbiotic supplementation in the CKD population and larger well-designed intervention trials are justified.[82]

In addition to commercially available nutrition supplements such as pre- pro- and synbiotics, other foods, nutrients and dietary patterns may modulate the gut microbiota and therefore influence health outcomes (Table 2). Historically, patients with CKD have been advised to follow restrictive dietary recommendations based on individual nutrients such as potassium, phosphorus, sodium and protein.[119] Not only do patients consider these recommendations complex and challenging to adhere to[5], but they are also likely to contribute to the altered microbiota and production of uremic retention solutes seen in this cohort of patients.[120,121] To this end, healthy dietary patterns such as the Mediterranean and DASH-style diet may be a strategy for CKD patients to modulate the gut microbiota. It is therefore encouraging that in recent years there has been a shift towards the recommendation of healthy dietary patterns; diets rich in vegetables, fruits, legumes and whole grains with lower consumption of red meat, sodium and refined sugars for patients with CKD.[122] Whilst limited in the CKD population, there are studies that confirm that healthy dietary patterns have a positive impact on gut health through their increased fibre intake and reduction in red meats, resulting in increased prevalence of beneficial bacteria (.*Bifidobacterium*, *Lactobacillus* and *Eubacterium spp.)* and lower levels of proteolytic bacteria.[123,124] Whilst healthy dietary patterns represent an ideal dietary approach for patients with CKD, in part due to the associated lower risk of mortality[125], their effects on the gut microbiota needs to be further explored.

The role of the gut microbiota in human health and disease has advanced greatly, facilitated by recent technological developments. The advent of NGS is promising to transform our approach to traditional nutrition interventions, with the gut microbiota emerging as a tool for personalised treatment strategies. The ultimate aim of precision medicine is to integrate an individual’s genome, environment and lifestyle to determine the best approach for the prevention or treatment of disease.[136] Personalised formulations of pre-, pro- and synbiotic supplements that can be used to modulate the gut microbiome represent an intriguing personalised nutrition strategy. However, it remains unclear whether these interventions lead to improvements in clinical outcomes for patients with CKD.[82]

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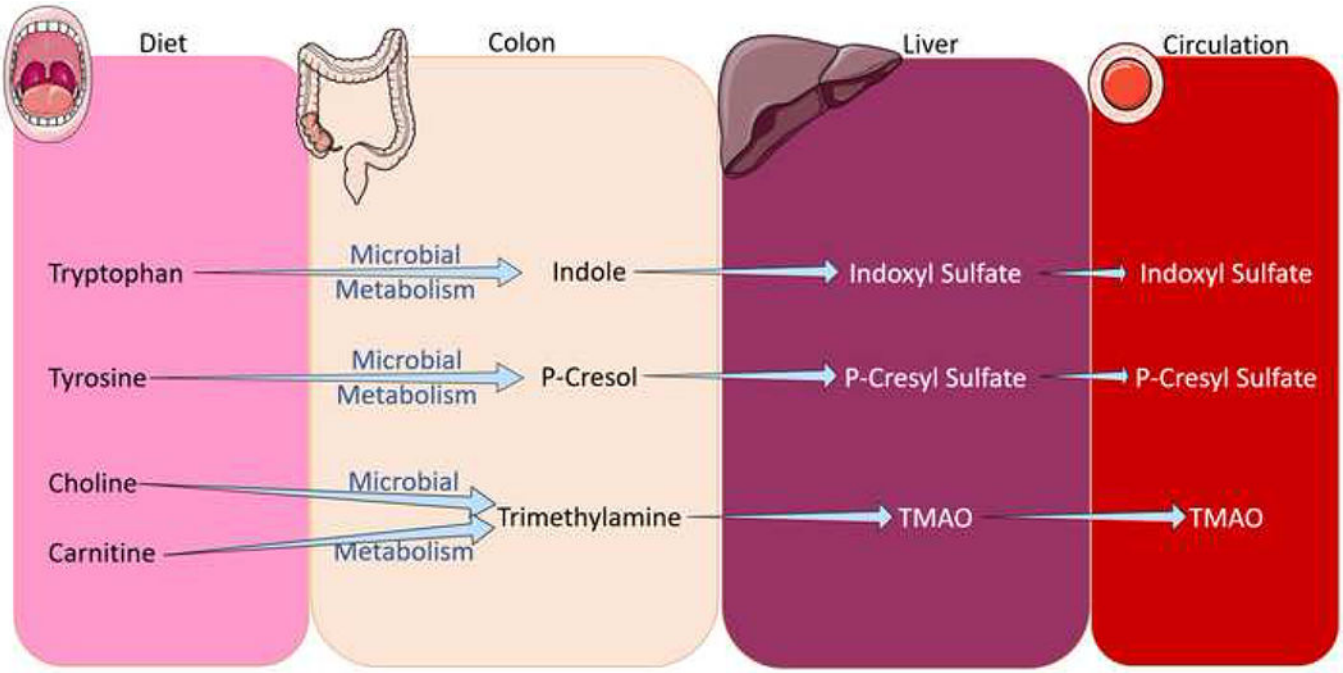

Figure 1:
Common dietary components that undergo microbial metabolism in the colon, leading to the formation of uremic retention solutes which exhibit detrimental effects in CKD. TMAO = trimethylamine-N-oxide.

Table 1.

A glossary of key terms related to microbiota and kidney disease

Microbiota	Microbiota refers to a collection of microorganisms that are found in a particular sample or location. Whilst the term microbiota may be used to refer to all microorganisms; bacteria, bacteriophage, viruses, fungi and protozoa, it is commonly used in reference to bacterial communities. When the term microbiota is preceded by a location, it is referring to all the microbes in a specific environment. For example 'gut microbiota' refers to the microbial community of the intestinal tract.
Microbiome	The collective genetic material of the microbiota is called the microbiome.
16S rRNA sequencing	16S rRNA sequencing identifies bacteria by sequencing a portion of the bacterial 16S rRNA gene. 16S sequencing provides greater insights into the bacterial community than could be achieved by culture-dependent methods, however there are limits in terms of the depth and resolution that can be achieved.
Shotgun sequencing	Shotgun sequencing, also known as metagenomics, involves the sequencing of all microbial DNA within a sample. Metagenomic shotgun sequencing provides the most informative view of the microbiome, however generates vast volumes of data, is quite computationally intensive and requires specialised bioinformatics skills.
Metatranscriptomics	Metatranscriptomics involves the sequencing of total microbial mRNA to provide insight into the functional impact of the microbiota.
Metaproteomics	Metaproteomics involves identifying all microbial proteins in a sample which can provide insight into the function of the microbiota.
Metabolomics	Metabolomics involves identifying a set of metabolites within a sample. The measurement of microbially produced metabolites can provide insight into the functional impact of the microbiota on the host.
Prebiotics	Prebiotics are dietary components that are selectively utilised by certain microorganisms to confer health benefits to the host. Examples of prebiotics include; fructooligosaccharides, galactooligosaccharides and inulin.
Probiotics	Probiotics are live beneficial bacteria which when administered to the host in adequate amounts are beneficial to health.
Postbiotic	Postbiotics are the metabolic byproducts from probiotic microorganisms that may have biological activity in the host.
Synbiotics	The term synbiotic refers to the co-administration of a pre- and a probiotic.
Symbiotic	Not to be confused with synbiotic. A symbiotic relationship refers to a long-term, biological interaction between two or more different organisms that are living in close physical association
Uremic Retention Solutes	Uremic retention solutes are metabolites produced as end products of microbial metabolism that accumulates in circulation in patients with chronic kidney disease.

Table 2:

Dietary components, their effect on the gut microbiota and evidence in chronic kidney disease

Dietary component	Effect on gut microbiota	Evidence in CKD
Fermented foods (e.g., sauerkraut, kimchi, kefir, kombucha, tempeh, natto, miso)	Fermented foods contain potentially probiotic organisms such as *Lactobacillus* and *Bifidobacterium spp.* Potential benefits include; • Competitive colonisation[122] • Antimicrobial production ↑ intestinal transit time ↑ intestinal pH[122] • Production of immune-regulatory by-products[130]	No known trials in the CKD population however may be a strategy to modulate gut microbiota.
Polyphenols (e.g., coffee, tea, cocoa, berries, plums, nuts, artichokes)	Two-way relationship between polyphenols and the gut microbiota; • polyphenols modulate the gut microbiota through competitive colonisation[131] • Biotransformation of polyphenols by the gut microbiota alters their bioavailability[132]	No known trials in the CKD population. The biotransformation of polyphenols by the gut microbiota and subsequent alteration in bioavailability potentially reduces markers of cardiovascular risk.[132]
Low FODMAP diet	A low FODMAP diet results in a decreased concentration of *Bifidobacteria*[133] and increased concentrations of *Roseburla* and *Ruminococus*.[134]	No known trials in the CKD population however may improve gastrointestinal symptoms of IBS.
Artificial Sweeteners	Artificial sweeteners induce altered gut microbiota with an increased abundance of *Enterobacteriaceae, Bacteroides* and *Clostridium*.[135]	No known trials in the CKD population, however artificial sweeteners may induce glucose intolerance and have been correlated with markers of metabolic syndrome.[135]