Mechanisms of the intestinal and urinary microbiome in kidney stone disease

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Abstract

Kidney stone disease affects ~10% of the global population and the incidence continues to rise owing to the associated global increase in the incidence of medical conditions associated with kidney stone disease including, for example, those comprising the metabolic syndrome. Considering that the intestinal microbiome has a substantial influence on host metabolism, that evidence has suggested that the intestinal microbiome might have a role in maintaining oxalate homeostasis and kidney stone disease is unsurprising. In addition, the discovery that urine is not sterile but, like other sites of the human body, harbours commensal bacterial species that collectively form a urinary microbiome, is an additional factor that might influence the induction of crystal formation and stone growth directly in the kidney. Collectively, the microbiomes of the host could influence kidney stone disease at multiple levels, including intestinal oxalate absorption and direct crystal formation in the kidneys.

The human microbiome comprises all of the microorganisms that live within as well as on the human host[1,2]. One of the most frequently studied microbiome sites in the human host is that of the intestine[3], which acts as an effective metabolic organ[4,5] and has been shown to have important roles in the maintenance of overall human health by not only being critical for the digestion of food and extraction of nutrients but also by regulating the immune response of the host[6], preventing overgrowth of pathogens[7], regulating cell proliferation and vascularization of the host[8,9], and regulating endocrine functions of

the intestine[10], as well as via neurological signalling[11], regulation of energy[12], and by regulating the overall metabolism of the host[13,14]. Disruption in the composition of the microbiome — termed 'dysbiosis' — contributes to several disease states within and distant from the intestines[15] including inflammatory bowel disease (Crohn's disease and ulcerative colitis)[16-18], asthma[19], obesity[20,21] and type 2 diabetes[22,23], as well as behavioural disorders including anxiety and depression via the gut–brain axis[24-26]. In general, dysbiosis can be driven either by the loss of health-protective bacteria, such as members of the *Lactobacillus*, *Faecalibacterium*, *Coprococcus* and *Ruminococcus* species, or by the acquisition and/or overgrowth of disease-causing bacteria such as *Clostridium difficile*, which are termed loss-of-function and gain-of-function dysbiosis, respectively[27].

Intestinal dysbiosis and oxalate homeostasis.

Urinary oxalate, which is derived from both exogenous and endogenous sources, contributes to the formation of 70–80% of kidney stones, and the urine of stone formers is frequently more supersaturated with oxalate than that of healthy subjects[28,29]. Oxalate-metabolizing bacterial species (OMBS) in the gut have been speculated to have an active role in sustaining oxalate homeostasis by reducing the amount of dietary oxalate absorbed. This mechanism is useful in the scenario of high dietary oxalate intake (the primary focus of this Review) and potentially also by sequestering circulating oxalate into the digestive tract, which could be particularly useful in individuals with high endogenous oxalate production[30-33].

Oxalate-degrading microorganisms can be categorized into two groups: obligate oxalate consumers, such as *Oxalobacter formigenes*, that require oxalate as a carbon and energy source[30,34]; and facultative oxalate consumers, which include species from the *Lactobacillus, Bifidobacterium, Enterococcus, Clostridium, Eggerthella, Providencia, Streptococcus* and *Leuconostoc* genera[35], which degrade oxalate when present but often exhibit growth inhibition with oxalate exposure[36-38]. Attempts to introduce oxalate-degrading microorganisms into the digestive tracts of humans or rodents have typically resulted in an ephemeral decrease in urinary oxalate excretion, with values returning to baseline in as little as 5 days[39]. In fact, of 14 clinical studies to date that used either *O. formigenes* or various *Lactobacilli* as probiotics, only 8 showed varying significant effects of treatment on urinary oxalate levels[33,36,40-51]. Of the three studies that quantified persistence of the oxalate-degrading function, values of persistence ranged from <2 weeks to >4 months[33,42,49]. In the three randomized, double-blind, placebo-controlled clinical studies using *O. formigenes,* the probiotics showed no effect on urinary oxalate excretion[40,41,43]. However, these clinical trials were notably conducted only on patients with primary hyperoxaluria, meaning that probiotic supplementation of *O. formigenes* might still be effective in patients with enteric hyperoxaluria. That said, based on the data available to date, our overall understanding of the oxalate-degrading properties of the gut microbiome remains incomplete.

The inconsistency of the results from clinical studies that sought to reduce urinary oxalate through the introduction of specific OMBS into the gut might be partly explained by the heterogeneous aetiology of hyperoxaluria. Individuals whose hyperoxaluria is caused by primary hyperoxaluria, for example, would be unlikely to benefit from enhanced

gut microbial oxalate degradation because the source of oxalate in these patients is the liver[52]. However, regarding patients whose hyperoxaluria arises largely from dietary oxalate absorption and who should, therefore, benefit from increased levels of OMBS, results from metagenomic and animal studies shed light on potential reasons for inconsistent findings. Several metagenomic studies published since 2016 have used comparative 16S rRNA metagenomic sequencing to identify differences in the gut microbiome between patients with an active episode of kidney stone disease and those with no history of stones[53-59]. Although differences in gut microbiome composition were apparent between groups, particularly with regard to the *Bacteroides, Ruminococcus, Coprococcus, Prevotella,* and *Oscillospira* genera, none of the studies reported a difference in *O. formigenes*. However, one shotgun metagenomic study reported significantly lower diversity in oxalate-degrading genes in the population of patients with a history of kidney stones ($P = 0.002$)[57], whereas another study showed that this population exhibited lower levels of bacterial taxa that are stimulated by dietary oxalate[58]. Together, these metagenomic studies suggest that some level of metabolic redundancy and/or cooperation within the microbiome help to maintain oxalate homeostasis, rather than a single or a few specific OMBS, and that the loss of these microbial consortia might increase the risk of the development of stones. This hypothesis is supported by animal studies in which faecal transplants from wild animals resulted in persistently lower urinary oxalate levels, the effect of which was greater than from oral probiotics of mixed oxalate-degrading bacteria; however, this effect was eliminated by antibiotics use or a high-fat, high-sugar diet, suggesting that the fine-tuned microbial consortia that convey maximal oxalate degradation can easily be disrupted by various external factors, resulting in increased oxalate absorption and a corresponding increased risk of stone development[60-62].

Beyond oxalate, one other animal study in rats found that the gut microbiome broadly affected numerous kidney stone risk factors[63]. Specifically, urinary calcium was decreased by 55% following faecal transplant ($P < 0.001$), whereas urinary oxalate levels were 24% lower than baseline levels ($P < 0.01$). Furthermore, pH increased by 0.6 units from the baseline level of 5.85 ($P < 0.001$), and a 29% increase in gastrointestinal alkali absorption ($P < 0.001$) was observed as well as 90% increased expression of Slc26a6 (anion transporter of chloride, oxalate, sulfate and bicarbonate) in the caecum following transplant.

Similarly, faecal transplant into germ-free mice led to decreased urinary calcium and oxalate and increased urinary pH[63]. All together, these findings suggest that intestinal microbiome composition might have an important effect on metabolism and urinary chemistries relevant to kidney stone disease risk. However, besides these studies, the role of the gut microbiome in risk factors beyond oxalate has been poorly explored to date.

Effect of urinary microbiota on kidney stone disease risk factors

In 2012, Wolfe and colleagues reported the discovery that the urinary tract is not sterile, even in the absence of any symptoms of a urinary tract infection[64], opening up a new avenue for research into the influence of the human microbiome on kidney stone disease.

Only two case–control studies have been published investigating the association between the urinary tract microbiome and urinary stone disease, both of which report significant assocations[59,65]. In a 2020 study, Xie et al.[65]. studied the urinary microbiome composition of men with calcium-based kidney stones and compared its composition with that of non-stone-forming men. The majority of participants were first-time stone formers (20 of 22, 90.9%) and only 2 were recurrent stone formers. Interestingly, the authors used urine samples taken from both the bladder and renal pelvis of participants and noted no significant differences in the urinary microbiome composition between the two sites in either patients or controls, suggesting that studying bladder urine (which is easier to collect than urine from the pelvis) is representative of the composition in the kidneys where stones form.

The 2019 study by Zampini et al. used metagenomic and metabolomic comparative analysis of the gut, urinary tract and stone microbiomes, and reported that the composition of the urinary tract microbiome could differentiate stone formers and non-stone formers more accurately than the gut microbiome[59]. Specifically, differential abundance analysis showed that only 1.9% of the operational taxonomic units identified in stool samples were significantly different in stone-forming patients and controls, whereas 8.8% of operational taxonomic units in the urinary microbiome were representative. In particular, Zampini et al. found the taxum *Lactobacillus* to be most abundant in the urine of healthy individuals, whereas members of the taxum Enterobacteriaceae were most abundant in the stone patient cohort. Thus, they concluded that the urinary microbiome might have an important role in the onset of kidney stone disease, and that the *Lactobacillus* genus and Enterobacteriaceae family could be important for the protection or promotion of the disease, respectively.

A number of studies have reported the presence of a consistent urinary microbiome among patients who formed specific types of stones[59,66-69], which suggests that bacteria might have a direct role in lithogenesis at the site of stone formation. Thus, investigators have employed different in vitro and in vivo study systems to understand the direct mechanistic role of urinary tract bacteria in the formation of stones. One study used a two-stage bioreactor that mimicked the kidney, ureter and bladder to investigate the role of *Proteus mirabilis* biofilm on struvite stone formation[70]. The developed model most closely mimics multiple factors affecting stone formation in vivo, including the natural migration of bacteria from a contaminated bladder vessel (that is, the site of bacterial inoculation) against urine flow (from the kidney to the ureter) and subsequent stone formation in the kidney. The researchers concluded that *P. mirabilis*-induced struvite stones were driven by microbial migration between the bladder and kidneys, bacterial attachment and biofilm formation, and increased urinary pH driven by ureolysis, all of which were followed by microcrystalline growth and aggregation, leading to stone formation[70].

The mechanisms by which urinary tract bacteria promote calcium oxalate (CaOx) stones has also been investigated. Studies indicate that *E. coli* are commonly found in CaOx stones and are present both in the centre and the periphery of the stones, suggesting that these microorganisms are not randomly entrapped into the stones but are actually associated with disease pathogenesis[59,68,69]. Although the exact mechanism has not been elucidated, these findings suggest that bacteria from the urinary tract could colonize or simply become trapped on the surface of the stone as it forms, acting as a nidus for

further crystal growth by providing additional attachment points for crystals. An in vitro study confirmed that intact and viable uropathogens, *E. coli, Klebsiella pneumoniae, Staphylococcus aureus* and *Streptococcus pneumoniae* all enhance CaOx crystal growth and aggregation[71]. Furthermore, a follow-up study by the same group found that the effects of intact viable *E. coli* to promote CaOx crystallization and aggregation were via the flagella[72], which potentially facilitate bacteria-assisted CaOx crystal growth and aggregation both indirectly, by promotion of bacterial biofilm formation, and directly by binding CaOx crystals via the dynamic charge of the flagella proteins.

Other components from the bacterial capsule, such as the lipopolysaccharide and the outer membrane vesicles, were found to promote crystal growth and aggregation but were not as potent as the flagella[72]. These in vitro studies are supported by data from Barr-Beare and colleagues[68] who showed that uropathogenic *E. coli* colonization and biofilm formation can induce substantial renal CaOx deposition in mice when uropathogenic *E. coli* was administered transurethrally at the same time as intraperitoneal glyoxalate (a precursor to oxalate)[68]. These data suggest that bacterial colonization and biofilm formation could have an important role in promoting CaOx crystal deposition and growth into larger stones.

The hypothesis that the urinary tract microbiome actively promotes lithogenesis is also supported by an interdisciplinary geobiology study that analysed thin sections of CaOx stones using a combination of optical techniques[73]. The images produced using this approach indicated that CaOx stones grow in a complex environment of biomass-rich nanolayers through a process of repeated dissolution, crystallization and remodelling, which the authors termed 'diagenetic phase transitions'. A number of different biomolecules have a role in driving these events and plausibly include biomolecules derived from a resident microbial community[66]. In fact, stone cultures have shown that nearly one-third of CaOx stones contained bacteria and up to 3% contained fungi[74]. The authors also observed that CaOx nanolayers are considerably smaller than the microbial layer that directly influences layering in other geological deposits, such as corals, caves and travertine marble on aqueducts. Thus, kidney stone biomineralization might be controlled, at least in part, by microbiome-derived biomolecules.

Similarly, a subsequent study analysed bulk-entombed DNA-sequenced fragments of CaOx, brushite and struvite stones from 11 recurrent kidney stone formers[75]. The analysis confirmed the presence of an entombed low-diversity community of bacteria and fungi including Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria and *Aspergillus niger*. Furthermore, in addition to the DNA sequence-based evidence, bacterial cells were also optically observed entombed and well preserved in amorphous hydroxyapatite spherules and fans of needle-like crystals of brushite and struvite[75]. Collectively, these data indicate that a 'microbiome' is incorporated into the CaOx, brushite and struvite stone formation processes. Although bacteria-associated urease activity is known to be the metabolic function driving infection stone formation[76], the mechanisms of how microbiome-associated metabolic functions drive lithogenesis of CaOx and brushite stones remains to be elucidated. One possibility is that CaOx and brushite stone-associated microbiomes might be merely bystanders in the urinary microbiome that become incorporated into the stone matrix as

the stone forms. However, given the symbiotic nature of bacterial microbiomes, this simple explanation is unlikely to represent the whole story.

The role of microbial metabolites in stone formation

Metabolites are the end product of a cascade of metabolic processes induced by the interaction between an organism and external factors that starts with the genome, and progresses through translation, protein synthesis and finally metabolism.

Thus, as the main communicators produced by the intestinal microbiome, metabolites are often at the intersection between host–microorganism and microorganism–microorganism interactions, such as those that promote kidney stone formation as they interact with and activate various host and microbial pathways involved in the maintenance of overall health[77,78]. Short-chain fatty acids (SCFAs), for example, are the main metabolites produced by the intestinal microbiome[79], and have been shown to have important effects on the metabolism of the host and determinants of health and disease[80]. Of particular relevance to kidney stone disease, SCFAs such as butyrate substantially affect gut health characteristics, including tight junction expression, transepithelial resistance and the expression of transporters that affect the absorption of compounds and/or ions relevant to kidney stone formation, including sodium, calcium and oxalate[81-85].

Previous metabolomic evaluations of the urine of stone formers have shown that metabolites involved in the processes of cell injury, such as lactate, are elevated in individuals with kidney stone disease[86]. Among the metabolites identified, some bacteria-derived biomolecules are associated with the promotion or inhibition of stone growth[59]. Among these, hippurate, a metabolic marker of microbiome diversity[87], was elevated in healthy individuals compared with those with stones[86] and also correlated negatively with kidney stone disease in a metabolomic analysis of urine samples from patients with and without kidney stones[88]. However, the mechanisms associated with these compounds in inhibiting stone formation require further study.

SCFAs, including acetate, propionate and butyrate, are the main metabolites produced by gut microbiota in the colon as a result of fermentation of dietary fibre and digestion-resistant starch[79], and are involved in the maintenance of intestinal epithelial health and function (FIG. 1). SCFAs in the lumen of the intestine are absorbed quickly by the duodenal mucosa through the electrogenic high-affinity transporter sodium-coupled monocarboxylate transporter 1 (SMCT1) and slowly by low-affinity SMCT2 in the jejunum or ileum[89]. Once absorbed, SCFAs are converted into either acetyl-CoA or propionyl-CoA, both of which are used to generate energy for intestinal epithelial cells via the tricarboxylic acid cycle[90]. As each butyrate molecule generates more ATP molecules than the other SCFAs (27 for butyrate versus 18 for propionate and 10 for acetate), butyrate is the main energy source for colonocytes, providing at least 60–70% of the energy required by these cells[91] (FIG. 1).

Although a role for these important bacterial metabolites has been established in conditions involved in metabolic syndrome, including obesity, high lipid levels, and diabetes[12,92-96], early evidence suggests that similar roles exist in the gut–kidney axis. For instance, some

SCFAs have been shown to be able to regulate renal dysfunction in acute and chronic kidney disease via activation of anti-inflammatory responses[81,97-102]. For instance, SCFAs decreased oxidative stress, the maturation of dendritic cells and their ability to induce $CD4^+$ and $CD8^+$ T cell proliferation, all of which are hallmarks of the inflammatory process leading to acute kidney injury[97]. Furthermore, sodium butyrate attenuated kidney dysfunction and tubular damage, probably because of the reduced expression of ILN6 and NF-κB, both key cytokines involved in the inflammatory process leading to acute kidney injury[98]. Lastly, both acetate and butyrate have been shown to protect against kidney injury by regulating reactive oxygen species (ROS) production by enhancing superoxide dismutase and catalase activities, which are responsible for breaking down ROSs[103]. Overall, these data suggest a direct effect of SCFAs produced in the intestine on the kidney via activation of G-protein-coupled receptors after intestinal absorption into the circulation[104]. Given that a role for ROS and inflammation in the kidneys has been suggested in the initiation of kidney stones[105,106], SCFA concentrations and intestinal absorption might affect specific inflammatory events in the kidney that could influence kidney stone formation. This area warrants further investigation.

Given the association between CaOx stone disease and intestinal oxalate absorption, the availability and absorption of SCFAs as the main determinants of overall intestinal epithelial health and function might have a direct role in the development of hyperoxaluria via direct effects on intestinal ion transport. For instance, SCFAs have a role in restoring and maintaining normal intestinal barrier function by affecting the expression of tight junction proteins to regulate paracellular permeability and solute transport via channels between cells (FIG. 2). Of all SCFAs, butyrate is the most important regulator of tight junctions by altering the expression of genes encoding tight junction proteins such as claudin-1 and zonula occludens 1 (ZO1) and by regulating the distribution of occludins[107,108]. In the context of kidney stone disease, decreased tight junction protein expression results in a more permeable gut barrier that enables increased absorption of relevant ions and/or compounds such as oxalate.

Butyrate has also been found to have a direct effect on transporters involved in transcellular transport of oxalate across the intestinal epithelium. Butyrate was shown to stimulate SLC26A3 expression and promoter activity via regulation of specific transcription factors, including yin yang 1 and GATA[109]. *Lactobacillus acidophilus* was also found to stimulate the expression of SLC26A3 via a transcriptional mechanism, which is interesting as this suggests a direct effect of bacteria on transporter expression[110]. These data suggest that butyrate producers and butyrate itself could have direct effects on transporters involved in oxalate transport across the intestinal epithelium. In this context, the levels of butyrate producers such as *L. acidophilus* and butyrate itself in the intestines of recurrent kidney stone formers might affect the expression and activity of transporters directly involved in intestinal oxalate absorption leading to a higher propensity to develop stones.

Butyrate was also found to have an important role in increasing transepithelial electrical resistance (TEER) via the activation of AMP-activated protein kinase (AMPK)[91,111], which further supports its effect on both paracellular and transcellular ion transport. TEER is the measure of the net movement of all ions across the intestinal epithelium and reflects

the resistance that the epithelium exerts against the movement of ions[112-114]. Increased permeability and overall 'leakiness' of the epithelium results in reduction of TEER and easier passage of ions. Na^+ and Cl^- are the most common physiological ions and drive the current that determines TEER; given that butyrate enhances colonic NaCl absorption via NHE2 and NHE3 transporters[112-114], that butyrate levels affect TEER is not surprising[115].

Evidence supporting a role for intestinal barrier function in recurrent CaOx kidney stone disease comes from the fact that dietary oxalate is absorbed via both paracellular and transcellular mechanisms, as the intestinal barrier is a determinant of the absorption of compounds and ions across the intestinal epithelium[84,116]. Studies involving non-stone-forming individuals have shown that ~5–10% of soluble oxalate is absorbed at the intestinal level (inter-individual and intra-individual variability 1–20%)[117]. However, intestinal oxalate absorption was found to be higher in stone-forming individuals, suggesting that intestinal integrity and function has an important role in increased oxalate absorption in stone formers. Given its role in maintaining overall intestinal epithelial health and function, butyrate produced by fermentation of dietary constituents by the gut microbiome might, therefore, also be involved in the overall maintenance of oxalate homeostasis and, therefore, CaOx stone risk.

Transcellular transport of oxalate is thought to occur via the activity of specific ion channels, mainly members of the SLC26 family of transporters (FIG. 3). SLC26A6 (also known as PAT1) and SLC26A3 (also known as DRA) have been shown to have key roles in regulating apical oxalate transport[116]. The corresponding oxalate transporter at the basolateral membrane remains unknown; however, SLC26A1 (also known as SAT1) is believed to be a candidate, based on studies in SAT1-KO mice that showed decreased intestinal oxalate secretion resulting in increased oxalate absorption[82].

In contrast to transcellular transport, paracellular transport involves the movement of oxalate between cells in response to transepithelial electrical and concentration gradients acting on oxalate, as well as membrane tight junction characteristics[118]. Interestingly, oxalate absorption (movement from the lumen of the intestine into the blood) occurs via both paracellular and transcellular pathways, whereas oxalate secretion (movement from the blood into the intestinal lumen) is believed to be mainly via the transcellular route. As a result, the overall net direction of oxalate movement across epithelia of the intestines is dependent on the relative contribution of each of the unidirectional fluxes and pathways[118].

Evidence has suggested that SCFAs might have a role in recurrent kidney stone disease, in particular in CaOx stones[119]. In a 2020 study, disruption of the intestinal microbiome of rats using antibiotics was shown to increase ethylene-glycol-induced crystal formation in the kidneys of the animals, which could be reduced by the administration of acetate, propionate and butyrate. Furthermore, studies in patients with recurrent CaOx stones showed that the intestinal microbiomes of patients were characterized by lower levels of SCFA-producing bacterial species and a lower abundance of genes involved in metabolic pathways associated with SCFA production compared with individuals without stone disease. Collectively, these data suggest that bacteria-induced SCFAs might have important roles in CaOx kidney stone disease.

Further evidence of a potential role for SCFAs in recurrent kidney stone disease comes from a case–control study of 88 children aged 4–18 years, including 44 patients with stones that contained at least 50% CaOx and 44 controls matched for age, sex and race[120]. Shotgun metagenomic sequencing revealed 31 bacterial taxa to be less abundant in the gut microbiomes of stone formers, which was reflected in the decreased abundance of the gene encoding butyryl-CoA-dehydrogenase (*bcd*), a key enzyme in butyrate metabolism. Overall, the study indicated that the loss of gut bacteria involved in butyrate production was associated with perturbations of the metabolome, suggesting that decreased butyrate production might have an important role in early-onset CaOx kidney stone disease.

Diet, microbiome composition and recurrent kidney stones

The human gut microbiome is largely — although not solely — influenced by diet. Nutrients are consumed in relatively large amounts multiple times a day, as they are required for hundreds of bodily functions including as substrates for the synthesis of proteins and other biologically active substances required for function and growth, co-factors for enzymes and structural components for bone, teeth and cell membranes[121]. Nutrients also regulate fluid and acid–base balance[122]. In addition to nutrients, food also provides non-nutrient substances that are absorbed and have systemic biological effects; these substances include phytochemicals, non-essential amino acids and fatty acids[123]. Plant-based foods, in particular, provide edible but indigestible carbohydrate, lignan and lignin components, which are collectively referred to as fibre. At various points along the gastrointestinal tract, different types of fibre (broadly grouped into soluble and insoluble) regulate bowel function, lipid metabolism and blood glucose levels[124]. As fibre is indigestible, it also reaches the lower intestinal tract to provide sustenance for gut microorganisms, the preponderance of which reside there.

Although fibre was traditionally the major dietary constituent thought to affect the gut microbiome, microbiome-modulating effects of other nutrients (including vitamins, minerals, protein and fats) and of non-nutrient compounds and chemicals such as polyphenols are now recognized[85,125]. Overall, all aspects of the human diet — not only fibre — seem to influence the gut microbiome in some way or another. As the effects of the gut microbiome on human health and disease are now known to reach far beyond the gastrointestinal tract, probably resulting from symbiotic interplay developed during human evolution[126], the optimal diet should, therefore, include foods that deliver sufficient energy and nutrients to maintain the host's homeostasis and also to optimize the function of the gut microbiome.

Role of diet in recurrent kidney stone disease.

Diet influences urinary tract calculus formation and growth at multiple points along the lithogenic continuum[83]. This influence is highly variable between individuals owing, in part, to variable intestinal tract digestive and absorptive function, underlying physiological and metabolic processes, food–drug interactions, and many other factors that potentially include variable gene–nutrient interactions and the expression of various diet-related diseases, such as type 2 diabetes mellitus[83]. Thus, the role of diet in kidney stone disease is complex, with

a plethora of primary dietary factors implicated in the most common types of kidney stones (TABLE 1). In susceptible individuals, dietary risk factors contribute to physiological and/or biochemical mechanisms that cause derangements in renal handling, leading to urinary stone risk factors and the potential to form kidney stones. Notably, kidney stones do not form in all people with dietary risk factors, suggesting that other factors combine with diet to contribute to stone disease. Given that the microbial composition of the human intestinal tract varies widely between individuals, the gut microbiome could be one of the factors that accounts for the variable incidence of stone disease among otherwise similar individuals.

Diet, microbiome, hyperoxaluria and CaOx stones.

Until ~2017, hyperoxaluria and CaOx kidney stones were the best known links between stone disease and the gut microbiome, owing to data showing that *O. formigenes*, an obligate oxalotroph common to mammals, was lacking in the faecal microbiomes of people who formed CaOx stones[30]. The lack of *O. formigenes* was thought to severely compromise bacterial oxalate degradation such that more dietary oxalate was absorbed and available to be excreted in urine. However, subsequent studies support the existence of oxalate-degrading networks comprising many different microorganisms, even in the absence of *O. formigenes*, which are responsible for most oxalate degradation[127,129]. This finding explains why not all individuals with absent or low *O. formigenes* form CaOx stones. The fact that, even among CaOx stone formers, hyperoxaluria is identified as causative in only 20–30% of cases[128]; means that other factors — such as hypercalciuria, hypocitraturia, hypomagnesiuria and/or low urine volume — must be involved.

Broader effects of diet on the human microbiome and kidney stones.

Assuming that hyperoxaluria caused by a lack of oxalate degradation in the human gut microbiome is only one of several influences on kidney stones exerted by gut microorganisms, this raises the question of additional pathways that might also be involved. This question is a focus of considerable research efforts and several groups are engaged in promising inquiries. Based on what is known about the effects of diet on the gut microbiome, coupled with known effects of the microbiome on human physiology, some inferences to kidney stones can be made.

First, given that diverse networks of microorganisms seem to be most responsible for oxalate degradation in the human intestinal tract[127,129], dietary factors associated with reductions or depletion in microorganisms known to inhabit these networks can be identified. For example, >100 bacterial taxa have been identified as being involved in oxalate degradation[128] and dietary factors that affect all of these microorganisms are not yet known. However, *Lactobacillus* and *Bifidobacterium*, two species with major roles in stone biology are both less abundant based on metagenomic studies in individuals who report increased salt (sodium chloride) intake[129]. Second, a reduction in oxalate intake, which is frequently recommended to patients with CaOx stones, promotes the depletion of OMBS, including non-oxalotrophs (bacterial species capable of breaking down and using oxalate as a main energy source)[128]. Although these changes might not be problematic for individuals who consistently follow a low-oxalate diet, transient consumption of oxalate could result in concomitant spikes in oxalate absorption and urinary excretion, potentially

stimulating CaOx crystal formation[130]. Moreover, the consequences of oxalate-degrading bacterial network depletion for other aspects of human health are unknown. Based on known effects of diet on the gut microbiome, and considering the physiological effects of changes in gut microbiota, several other speculative links between diet, the gut microbiome and kidney stone disease can be made (TABLE 2). For instance, the increased intake of saturated fats promotes colonization with species associated with decreased inulin sensitivity[131], resulting in a deficit in ammonia production and lower urinary ammonium excretion[132]. The resultant increase in urine pH leads to an increased risk of uric acid stone formation.

Although the role of diet in the gut microbiome is reasonably well understood, the effects of diet-induced gut microbiome profiles on kidney stone risk are relatively unknown. Dietary habits among stone formers have been investigated[129,133], but no studies have tested whether gut microbial modulation with diet affects stone risk or the incidence or recurrence of stone events. The effect of diet on the gut microbiome and its concomitant influence on kidney stone disease is, therefore, an emerging area of interest.

Antibiotics, dysbiosis and recurrent stones

In addition to diet, antibiotics use is also thought to be linked to an increased risk of kidney stone development. The discovery and administration of antibiotics is one of the most revolutionary advances in the history of medicine, enabling the treatment of bacterial infections and enhancing the safety of modern medical procedures. Thus, antibiotics are administered worldwide on a massive scale: in 2018, ~250 million courses of antibiotics were prescribed in the USA, equivalent to 763 antibiotics prescriptions per 1,000 persons[134]. In a given year, 30% of people are prescribed at least one antibiotic in the UK[135]. However, such widespread and frequent antibiotics use is not without risk: in addition to the well-established occurrence of antibiotics resistance, antibiotics use has been shown to be associated with alterations in microbiome composition.

In general, antibiotics-induced dysbiosis manifests as a reduction in the level of overall microbial diversity and as declines and expansions in the relative abundances of certain microbial taxa[136]. Antibiotics treatment affects a specific set of bacteria[137], most commonly resulting in increased abundance of Proteobacteria and higher ratios of Firmicutes to Bacteroidetes[138]. However, the specific pattern of microbiota alteration depends on the characteristics of both the antibiotic and the host. Antibiotics-related factors include antibiotics class, dosage, duration and administration route, and host-related factors include host age, lifestyle and microbiome composition[139]. Microbial alterations typically persist weeks to months after antibiotics exposure[138], but alterations lasting years have also been reported, particularly after administration of antibiotics with strong and broad activity against anaerobes. This effect is mainly because the natural re-introduction of these species into the intestinal environment after antibiotics treatment is limited owing to their high sensitivity to even low concentrations of oxygen[136].

Antibiotics exposure has been associated with several diseases, including asthma[140] and inflammatory bowel disease[141], and these associations are believed to be mediated by perturbation of the microbiome. The rapid shift in the epidemiology of kidney stone

disease suggests that environmental exposures, including antibiotics, could also be driving forces for the development of this disease. Research has, therefore, focused on identifying the relationship between antibiotics use and risk of kidney stone disease. For example, antibiotics use for 2 months was shown to be associated with a higher risk of incident kidney stones for women in early (age 20–39 years) and middle adulthood (age 40–49 years), compared with no antibiotics use[142]. However, the mechanism for this effect remains unclear, as only marginally lower urine pH and citrate values were observed among women with varying durations of antibiotics exposure[142].

Similarly, a separate study reported that adults with an active episode of kidney stone disease were more likely to have taken oral antibiotics in the year before diagnosis, compared with individuals who had no history of the disease[59]. The investigators also reported that antibiotics use had a considerable effect on the beta diversity (differences in bacterial species present in a given sample) of the urinary tract microbiome, but not the gut microbiome in this population. Finally, exposure to sulfonylureas, cephalosporins, fluroquinolones, nitrofurantoin/methenamine and broad-spectrum penicillins has been shown to be associated with an increased risk of kidney stone disease[143]. The magnitude of risk was greatest for exposure 3–6 months before diagnosis and for individuals exposed to antibiotics at younger ages. Although these associations were robust even when excluding individuals with previous UTI, the possibility of unmeasured confounding due to antibiotics prescribed for UTI symptoms without an associated diagnosis of UTI remains. Taken together, the findings of these studies do suggest that antibiotics use is a risk factor for kidney stone disease.

Nonetheless, the mechanisms by which antibiotics affect the gut microbiome in individuals with kidney stone disease is a critical knowledge gap. That antibiotics could alter the composition of the microbiome and metabolism of macronutrients is a plausible explanation — studies have shown that the use of certain antibiotics including chloramphenicol, colistin, doxycycline, erythromycin, polymyxin B, rifampin and tetracycline — markedly decreases the prevalence of colonization by *O. formigenes*, a major oxalate degrader in the gut[144]. Indeed, *O. formigenes* is susceptible to some of the same antibiotics that are associated with an increased risk of kidney stone disease, such as cephalosporins, nitrofurantoin and broad-spectrum penicillins[145]. Thus, persistent elimination of *Oxalobacter* after antibiotics exposure might be a risk factor and a mechanism resulting in CaOx kidney stone formation. However, given the complexity of the gut microbiota, decreased colonization by *Oxalobacter* alone is very unlikely to be responsible for incident kidney stone disease. Instead, an increased risk of stones might arise from dysfunction of bacterial networks.

For example, oxalate homeostasis is known to be maintained by a multispecies bacterial network that includes Ruminococcaceae and *Oscillospira*[129]. The bacteria in this network are believed to rely on the by-products of oxalate metabolism — CO_2 and formate — which can then be used in several metabolic pathways. Formate is used in most tissues in the synthesis of nucleotides and methyl groups[146]. In animal studies, exposure to antibiotics disrupts these symbiotic relationships that are critical for oxalate homeostasis and results in increased urinary oxalate levels and risk of CaOx stone formation[60].

Another possible mechanism is that antibiotics can select for multidrug-resistant bacteria, which might promote the growth of kidney stones via incorporation into the stone matrix as a component that facilitates crystal attachment and growth. This potential mechanism is supported by a study reporting that 70% of bacteria isolated from CaOx stones were resistant to multiple antibiotics[69].

Although antibiotic exposure might be an important link between dysbiosis and kidney stone disease, further studies are required to identify the specific perturbations of the gut and urinary microbiome caused by antibiotics. Potential research areas include identification of the way in which specific classes of antibiotics perturb the gut microbiome and which subgroups of patients are at an increased risk of kidney stones after antibiotics exposure. This information would have important implications for understanding the dramatic increase in the prevalence of kidney stone disease, revealing novel therapeutic pathways, and reducing inappropriate antibiotics use.

Effects of water intake on the intestinal microbiome

A strong correlation exists between low fluid intake and kidney stone formation[147]. As a result, all individuals, but especially stone formers, are encouraged to increase their fluid intake to ensure adequate hydration and normalization of urine parameters to within non-stone-forming ranges[147].

A 2020 systemic review studying the role of fluid intake in kidney stone prevention analysed the effect of six types of water and ten different types of juices on stone formation[147] and indicated that fluids low in calcium, as well as grapefruit, apple and orange juice, reduced urine CaOx saturations and subsequent stone formation.

The intestinal microbiome is thought to affect the way in which fluid intake influences overall health. For instance, a 2020 study used a novel approach to assess these effects, by comparing solute concentration measurements of domestic tap water with intestinal microbiome composition and health data from 85 monozygotic twins with existing faecal microbiota profiles[148]. The use of identical twins is particularly powerful in this study, as this approach controlled for genetic and early life factors, increasing the ability of the study to detect environmental effects. Overall, significant associations were observed between average daily doses of sodium in tap water and microbiota diversity. Furthermore, the overall microbial community composition differed with average daily doses of sulphate and chloride, and the abundance of taxa was associated with the chemical water composition in a number of participants.

A separate study similarly explored associations between source ($n = 3{,}413$ subjects) and intake ($n = 3{,}794$ subjects) of plain drinking water and gut microbiota composition. Overall, the source of drinking water — including bottled, tap, filtered or well water — was associated with significant variation in alpha and beta diversities. For example, the greatest differences in both alpha and beta diversity were observed between people consuming well water and individuals consuming bottled water. Interestingly, this study also noted differences in the relative abundance of specific bacterial taxa when comparing the intestinal

microbiome composition of individuals who consumed low (<1 l/day) versus high amounts (>1 l/day) of water, whereby those consuming <1 l/day tended to have a lower abundance of bacterial taxa, including Gammaproteobacteria, *Lactococcus*, Streptophyta, *Veillonella*, Lactobacillales, *Clostridium* and Erysipelotrichaceae, than those drinking >1 l/day.

Although these data are interesting, direct causality between decreased water intake and changes in gut microbiome composition has not been established. However, stool consistency — which is believed to be partly dependent on the degree of water intake — has been shown to be strongly associated with faecal microbial composition[148-150]. Of particular relevance to stone disease, constipation has been associated with a relative decrease in obligate bacteria, including *Lactobacillus, Bifidobacterium* and *Bacteroides* species, potentially influencing intestinal motility and secretory functions[151-154]. These particular bacterial species have the ability to degrade oxalate, which, in addition to regulating urinary saturation, might be another mechanism by which water regulates the risk of stone disease. These data emphasize the importance of incorporating water intake into studies on the role of the intestinal microbiome in kidney stone disease.

Future directions

Although research has begun to make clear links between the microbiome and the onset of kidney stone disease, more work is critically needed.

First, although microbial oxalate metabolism in the gut is clearly important in helping to prevent the formation of kidney stones, our understanding of the interactions between oxalate and the gut microbiota is limited. Second, the way in which gut microbiota contribute to other urinary abnormalities — such as imbalances in pH, uric acid, sodium, calcium, magnesium and other urinary risk factors — has received little attention, with the exception of studies that have found increased saturated fat intake to promote the abundance of bacterial species with decreased inulin sensitivity, hence decreasing ammonia genesis, leading to an overly acidic urine and increased risk of uric acid stone formation[131,132]. Finally, our understanding of the microbially derived proteins and metabolites that contribute to stone formation — either directly by promoting mineral crystallization and aggregation, or indirectly by inducing inflammation, oxidative stress or epithelial barrier function — is also very limited.

These gaps in knowledge can be addressed by performing long-term in vivo studies focused on assessing how changes in microbiome composition alter urine composition in patients and how varying oxalate levels affect the overall symbiotic nature of the urinary microbiome and associated overall metabolic function. That said, studies on how changes in intestinal and/or urinary microbiome compositions affect mineral crystallization and aggregation are more difficult to conduct, as current models of natural and spontaneous stone formation[155] include companion animals (dogs and cats), and various captive and wild species, including otters, dolphins and ferrets, that are limited by high costs, given the large number of animals needed to obtain reliable data, and ethical barriers preventing the use of such animals in research.

Future therapeutic potential.

Members of the intestinal microbiome regulate key physiological processes including protective, metabolic, trophic and immune functions[156-159]. The main function of bacteria in the colon is the fermentation of non-digestible carbohydrates to form SCFAs, which have a key role in regulating gut epithelial integrity and overall health. Given their direct role in the maintenance of processes that are determinants of overall health, the manipulation of the gut microbiome composition has considerable therapeutic potential as a 'natural' intervention to treat human diseases. Potential therapeutic approaches might include the introduction of individual bacterial species and/or networks whose loss is associated with a given disease, or even targeted eradication of bacterial species and/or networks identified as directly causing or exacerbating a given disease state.

Although these types of therapeutic approaches sound intriguing, several factors need to be taken into account before they can be implemented. For instance, although the re-introduction of bacteria seems relatively simple in theory, it can be very complex given that one is trying to re-introduce bacteria into an environment in which they did not survive to begin with and, therefore, a favourable overall environment might need to be created that will undoubtedly affect the composition of the pre-existing microbiome. Similarly, although targeted approaches to eradicate 'unwanted' bacterial species and/or networks sounds simple, the impact on innocent bystanders must also be taken into consideration. Additional research including these areas must, therefore, be conducted to better understand the potential overall effect of a given intervention before it can be translated into and implemented in clinical practice.

Conclusions

The current literature suggests an intriguing association between antibiotics-induced and probably also diet-induced dysbiosis of both the intestinal and urinary microbiomes and recurrent kidney stone disease. Ongoing work is focused on determining the exact mechanisms by which either of these microbiomes affects overall oxalate homeostasis. Although much focus has been on a direct role of members of the intestinal and urinary microbiome on oxalate breakdown, findings that SCFAs such as butyrate might have a role suggest the existence of oxalate-independent mechanisms, for example, via the regulation of immune responses in the kidney that are associated with kidney stone formation, gene and/or protein expression of relevant intestinal and kidney ion transporters, and/or the effect on actual stone growth via incorporation into the stone matrix.

The data collected to date suggest a multi-mechanistic role of both microbiomes in recurrent kidney stone disease and open up an exciting new area of research with the potential to lead to novel treatment options that can target multiple mechanisms of stone formation simultaneously, which is not possible with current treatment options that target a single mechanism.

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Key points

- Composition of the intestinal microbiome influences host metabolism and overall health.

- Patients with recurrent kidney stone disease have a disrupted intestinal microbiome composition, including reduced overall abundance of butyrate-producing species.

- Butyrate is a key short-chain fatty acid responsible for overall intestinal epithelial integrity and health, a major determinant affecting the absorption of oxalate and other ions relevant to kidney stone disease.

- Long-term history of antibiotic use is associated with an antibiotics-driven shift in intestinal microbiome composition and increased risk of kidney stone disease.

- Members of the microbiome have a role in different processes of stone formation, including intestinal oxalate absorption and crystal formation.

- Urine also has a native urinary microbiome, the composition of which is better able to differentiate between stone and non-stone formers than that of the intestine.
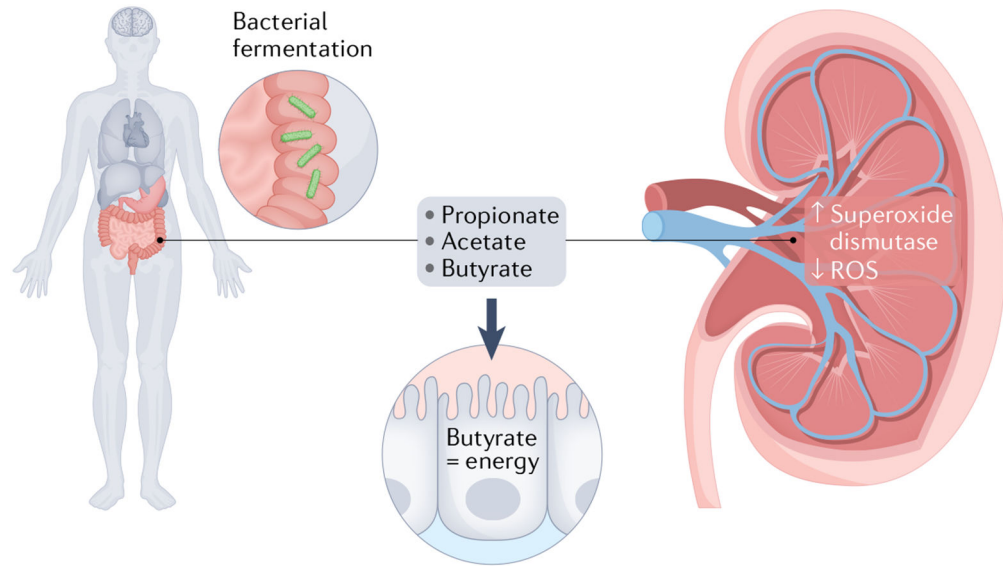
Fig. 1 |. The effect of short-chain fatty acids on mechanisms that might affect oxalate absorption and mechanisms of stone formation.

Short-chain fatty acids (SFCAs) are products of intestinal microbial fermentation of indigestible foods and include propionate, acetate and butyrate. These SCFAs are the main energy source of colonocytes, making them a key determinant of intestinal epithelial layer health. In addition, SCFAs in the gut have distant effects on the kidney, including the production of reactive oxygen species (ROS), which have been implicated in renal changes that trigger the initiation of crystal formation.
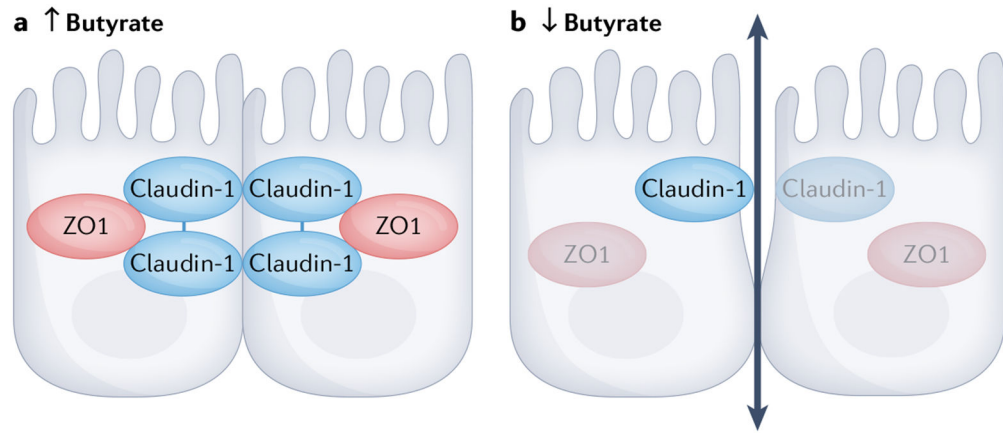
Fig. 2 |. The role of butyrate in regulating intestinal epithelial cell tight junctions.
Butyrate regulates the expression of genes encoding tight junction proteins, including claudin-1 and zonula occludens 1 (ZO1), and regulates the distribution of occludins. In the presence of high levels of butyrate, the expression of tight junction proteins and distribution of occludins is such that tight junctions are well formed. In an environment where butyrate levels are low, decreased expression of key proteins that form tight junctions causes the junctions to loosen and increases absorption.
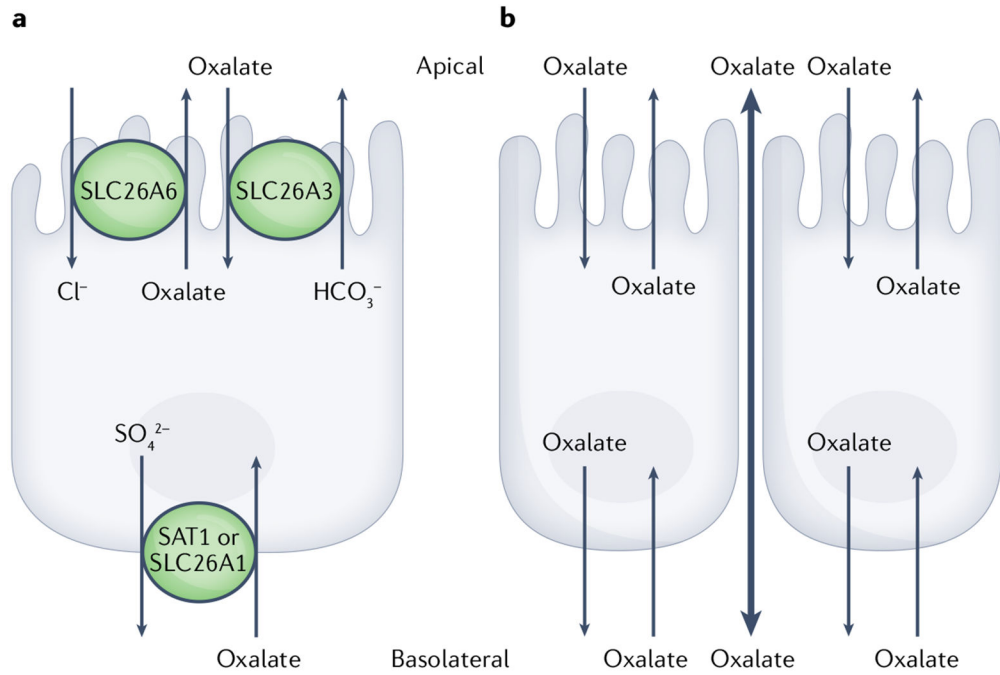
Fig. 3 |. Oxalate transport across intestinal epithelium.

Oxalate absorption and secretion across the intestinal epithelium occur via transcellular (through cells) and paracellular (between cells) mechanisms. Transcellular oxalate absorption and secretion involve transporters found on the apical (lumen of intestine) and basolateral (circulation) sides of the intestinal epithelial layer. For absorption, dietary oxalate enters intestinal epithelial cells on the apical side via SLC26A3 and exits into the circulation via a transport mechanism that remains to be elucidated, but might include SLC26A3. Secretion of oxalate from the circulation back into the lumen of the intestine occurs via SLC26A1 on the basolateral side and SLC26A6 on the apical side of the membrane. Paracellular transport of oxalate is passive and occurs through the tight junctions of the cells on the basolateral side via SLC26A3 and SLC26A6.

Table 1 |

Common dietary risk factors for calcium and uric acid stones

Dietary risk factor	Mechanism	Urinary aberration[a]	Specific stone risk
Fluid intake insufficient to maintain suitably low urine supersaturation	Concentrated urine	Increased urine supersaturation for crystal formation	All types of stones
Intake of bicarbonate precursors insufficient to compensate for acidogenic foods in diet	High dietary acid load inducing bone resorption and increased renal citrate reabsorption	Hypercalciuria Hypocitraturia Overly acidic urine	CaOx, CaPhos, uric acid
Excessive intake of salt (as NaCl)	Expansion of extracellular volume and decreased renal calcium reabsorption in nephron	Hypercalciuria	CaOx, CaPhos
Excessive calcium supplementation (above the DRI)[b]	Increased intestinal calcium absorption	Hypercalciuria	CaOx, CaPhos
Excessive vitamin D supplementation (above the DRI)[c]	Increased intestinal calcium absorption	Hypercalciuria	CaOx, CaPhos
Vitamin D intake insufficient to maintain normal limits	Secondary hyperparathyroidism and bone resorption	Hypercalciuria	CaOx, CaPhos
Calcium intake insufficient to meet needs for bone, especially in the context of increased dietary protein intake	Rise in calcitriol production	Hypercalciuria	CaOx, CaPhos
Calcium intake insufficient to compensate for oxalate load in the diet, especially in the context of malabsorption	Increased intestinal absorption of dietary oxalate	Hyperoxaluria	CaOx
Excessive vitamin C supplementation (>2,000 mg/day)	Increased biosynthesis of oxalate		
Excessive intake of animal-derived purines	Increased uric acid biosynthesis	Hyperuricosuria	Uric acid

CaOx, calcium oxalate; CaPhos, calcium phosphate; DRI, dietary reference intake. [a]Assessed using 24-h urine collection. [b]DRI for calcium is 1,200 mg/day for adults >19 years of age. [c]DRI for vitamin D is 600 International Units (15 mcg)/day for adults.

Table 2 |

Dietary factors influencing the gut microbiome, altering the risk of kidney stone disease

Increased dietary factor	Effects on gut microbiome	Potential implications for urolithiasis	Effect on risk of specific stone types
Fibre	Expands microbial diversity	Increases oxalate degradation capacity of microbial networks; enhances microbial production of short-chain fatty acids	Reduced dietary oxalate absorption and urinary excretion[a]	↓CaOx stones
Oxalate	Selects for oxalate-degrading bacteria	Increases oxalate degradation and promotes oxalate secretion into the digestive tract[160]	Reduced dietary oxalate absorption and urinary excretion[a]	
Legumes	Promote colonization of bacteria that produce short-chain fatty acids	Increases gut barrier[161] and reduces inflammation	Reduced dietary oxalate absorption and urinary excretion[a]	
Inulin-type fructans		Improves colonic mucosa absorptive capacity	Increased dietary magnesium absorption[162] and urinary excretion	
Fructo-oligosaccharides	Increase bacterial production of butyrate	Promotes skeletal resistance to parathyroid hormone[163]	Reduced urinary calcium excretion	↓CaOx stones ↓CaPhos stones
Various polysaccharides and disaccharides (e.g. inulin, lactulose)	Promote colonization of *Bifidobacterium longum* and other *Bifidobacteria*[164]	Reduces bone resorption, potentially by downregulating osteoclast activity[164]	Reduced urinary calcium excretion	
Phytoestrogens	Provide substrate for microbial production of equol	Equol binds to human oestrogen receptor to decrease bone resorption[165]	Reduced urinary calcium excretion	
Animal meat (including fish and seafood)	Selects for sulfidogenic bacteria	Increases bacterial production of sulphuric acid, leading to increased intestinal permeability[166]	Increased dietary oxalate absorption and urinary excretion	↑CaOx stones
Salt (sodium chloride)	Suppresses colonization of *Bifidobacteria* and *Lactobacillus*[129]	Reduces oxalate degradation capacity of microbiome	Increased dietary oxalate absorption and urinary excretion	
Saturated fats	Increase abundance of lipopolysaccharide-bearing bacterial species	Activates Toll-like receptors on immune cells and increased intestinal permeability and inflammation[167]	Increased dietary oxalate absorption and urinary excretion	
	Promote colonization of species associated with decreased insulin sensitivity[131]	Promotes defect in ammonia genesis leading to lower urinary ammonium excretion[132]	Overly acidic urine	↑Uric acid stones

The listed effects do not account for synergy between dietary factors; the impact of any one dietary factor is likely to be less important for the gut microbiome than the dietary pattern as a whole. CaOx, calcium oxalate; CaPhos, calcium phosphate. [a]Assumes that higher oxalate consumption promotes concomitant increases in oxalate-degrading bacteria.