

Vitamins as regulators of calcium-containing kidney stones – new perspectives on the role of the gut microbiome

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Abstract

Calcium-based kidney stone disease is a highly prevalent and morbid condition, with an often complicated and multifactorial aetiology. An abundance of research on the role of specific vitamins (B₆, C and D) in stone formation exists, but no consensus has been reached on how these vitamins influence stone disease. As a consequence of emerging research on the role of the gut microbiota in urolithiasis, previous notions on the contribution of these vitamins to urolithiasis are being reconsidered in the field, and investigation into previously overlooked vitamins (A, E and K) was expanded. Understanding how the microbiota influences host vitamin regulation could help to determine the role of vitamins in stone disease.

Sections

Introduction

Vitamin A

Vitamin B₆

Vitamin C

Vitamin D

Vitamin E

Vitamin K

Future directions

Conclusions

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Introduction

Kidney stone disease affects ~0.2–20% of the world's population and places a considerable clinical and economic burden on global health systems^{1–3}. In North America, ~10% of people will have experienced a kidney stone episode in their lifetime^{4–6}. Urinary stone disease is also a highly recurrent condition, with up to 50% of patients developing a recurrent stone in the absence of preventative treatment⁷. Modern medical treatment is available, but kidney stones still result in substantial patient morbidity, including pain and renal colic, chronic kidney disease, infections and urosepsis⁸. Consequently, the annual cost of direct care to treat patients with nephrolithiasis exceeds \$10 billion in the USA alone⁹. Amongst patients with stone disease, 3.1 million workdays are estimated to be lost to hospitalizations and ambulatory care, further contributing to the financial burden indirectly caused by this disease^{10,11}. The prevalence of the disease is expected to inflate owing to the increased global incidence of nephrolithiasis; thus, additional research focused on disease aetiology is needed, with the aim of mitigating patient morbidity and associated expenses^{1,6,12,13}.

The largest proportion of kidney stones contain calcium oxalate (60–75%) or calcium phosphate (15–20%)^{4,14,15}. Other less common stone compositions include uric acid, struvite and cystine^{4,14,15}. The formation of renal calculi is not completely understood, but is predicted to proceed along the path of crystal nucleation, growth, aggregation and fixation within the kidney, where the crystal can further grow into a macroscopic stone¹⁶. The presence of stone-forming substrates or inhibitors at any point in this process can promote or hamper crystal formation¹⁷. Randall's plaques or plugs – which are calcium phosphate deposits found in the papillary interstitium or collecting ducts, respectively – are hypothesized to serve as a nidus for crystal attachment and subsequent stone development¹⁸. Additionally, the components of these deposits can induce reactive oxygen species (ROS) and inflammation, which damage the epithelium and promote further crystal deposition – a process that is probably accelerated by crystalluria¹⁹.

Diet has long been a risk factor for stone disease^{20–22}. Results from empirical analysis showed that the typical Western diet produces a urine chemical profile that is more susceptible to calcium-based stone formation than that obtained with other nutritional regimes such as an ovo-lacto-vegetarian diet²³, which is in part owing to decreased urine volume, pH and citrate levels. Western diet is characterized by increased consumption of animal protein, saturated fats and refined sugars, but reduced consumption of fruits and vegetables²⁴. In turn, this diet shifts the gut microbiota to a predominantly proteolytic state, which increases uraemic toxin²⁵ and endotoxin²⁶ production. Together, these factors contribute to increased inflammation and to the development of metabolic diseases such as irritable bowel disease^{24,27}.

Notably, the Western diet is depleted in dietary vitamins²⁸. Vitamins have a long and complex history in kidney stone management, with some of the earliest reports on this topic dating back to the middle of the twentieth century²⁹. Since then, numerous large epidemiological datasets such as the Nurses' Health Study I and II and Health Professionals Follow-up have been analysed to assess the role of vitamins in stone disease, but with inconclusive and often conflicting results. The lack of consensus among different studies exemplifies the complicated nature of the relationship between stone disease and vitamin intake, which can be further complicated considering the role of the microbiota.

The potential role of the gut microbiota in stone disease is a relatively new research line³⁰. The idea of a role of gut microbiome in stone disease is supported by the strong association between oral antibiotic use and incidence of kidney stones³¹. The initial attempts

to mechanistically understand how bacteria influence nephrolithiasis focused on *Oxalobacter formigenes*, a Gram-negative bacterium that uses oxalate as the sole carbon source³². Results from an early study showed that the presence, whether indigenous or supplemented (for example, as a probiotic), of *O. formigenes* reduced urinary oxalate levels and could provide a protective effect against calcium oxalate stone formation³³. However, this conjecture was challenged by results from subsequent studies showing that there was no difference in the abundance of *Oxalobacter* between stone formers and healthy individuals^{34–38}. Instead, this bacterium might be part of a network of bacteria involved in host oxalate homeostasis^{39–41}.

In subsequent studies, the role of gut microbiota as a whole has been assessed in stone disease, rather than focusing on individual bacterial types. In general, the gut microbiota of stone formers has repeatedly been shown to differ from that of non-stone-forming individuals, but the specific differences are often not consistent across studies^{35–37,40,42–44}. Results from a 2020 study showed that the level of evidence for these differences varies according to bacterial taxa, but the microbiota of stone formers is likely to be depleted in short-chain fatty acid-producing bacteria and to have an elevated amount of bacteria associated with inflammation^{38,45}. Results from a meta-analysis³⁴ showed that the gut microbiota from stone formers had lower diversity than that of healthy individuals, but specific bacterial taxa that might be crucial in influencing stone disease were not identified. Additionally, stone composition was associated with the urinary tract microbiome but not with the gut microbiome; thus, the authors concluded that, based on this analysis, the urinary microbiome might influence the host environment to promote or inhibit stone formation³⁴. In this report, some knowledge of the role of gut microbiome in stone disease is provided, but functional changes in bacteria metabolism that could differ between different species and strains in the gut were not fully considered. These functional changes in the microbiome are poorly captured by traditional 16S rRNA sequencing methods and are best addressed by shotgun metagenomic sequencing and metabolomics³⁸.

The effect of nutrient intake on gut microbiota composition and the gut–kidney axis has also been studied³⁹. Interestingly, all vitamin synthesis pathways have been identified in human faecal samples through shotgun metagenomic sequencing, suggesting that the microbiota might have some roles in vitamin acquisition by the host, including vitamins that were previously not considered as microbially derived⁴⁶. Not all bacteria can produce these vitamins *de novo*, but in an adapted microbial network, bacteria might exchange resources in the extracellular “pantryome”⁴⁷ to complete gaps in metabolic pathways. The “pantryome” concept is based on the idea that individual bacteria that lack some nutrients can take these nutrients from a pool of metabolites shared with other members of the microbiome and, in return, donate the excess metabolites back to the pool for other members to use. Metabolite sharing enables individual members of the gut microbiota to make up for functional absences in the metabolic pathways. Thus, an individual bacterium might not directly correlate with a disease state – as perceived from traditional 16S rRNA sequencing studies – and instead, the function of the entire community as a whole should be considered.

The contribution of the microbiota to vitamin absorption and activity has been overlooked in many current studies in which the role of the microbiota has been assessed in several diseases⁴⁷. Gaps in the understanding of the role of vitamins and kidney stones have become particularly evident considering the uncertain history of vitamins and

kidney stone disease, and the evidence that gut bacteria are able to synthesize these vitamins^{47,48}.

In this Perspective, we discuss the potential roles of vitamins in stone disease (Box 1). Specifically, we focused on vitamins A, B₆, C, D, E and K, considering the suspected roles of these vitamins in stone formation and potential modulation by the gut microbiota. We also provide a new perspective on the role of the microbiota as a local producer of vitamins and an overall regulator of vitamin bioavailability and function (Fig. 1).

Vitamin A

Vitamin A is the generic descriptor for a group of lipid-soluble retinoids that are recognized by a β -ionone ring with an isoprenoid side chain⁴⁹ (Fig. 2). Vitamers of vitamin A are named according to the functional group at the end of the isoprenoid chain. Dietary sources of vitamin A exist in the form of provitamin A (such as carotenoids) or preformed vitamin A (such as retinol and retinyl esters), which are obtained from the consumption of pigmented vegetables and animal products, respectively⁵⁰. In the body, these substances are metabolized into the active forms of vitamin A, retinal and retinoic acid, by enzymes present in the intestines and liver⁵⁰.

In rats, vitamin A deficiency has been shown to reduce urinary citrate and pH, and to increase the presence of urinary calculi^{51,52} (Table 1). Supplementation with high amounts of vitamin A (retinol) in a calcium oxalate stone rat model enhanced urinary citrate levels, increased urinary pH, and lowered the concentration of calcium and oxalate in renal tissues⁵³. These changes observed in vitamin A-supplemented rats could be partially attributed to the ability of vitamin A to alter gene expression in cells in the collecting ducts, which have crucial roles in maintaining urinary pH⁵⁴. The influence of vitamin A on urinary calculi formation has been corroborated by the evidence that vitamin A deficiency resulted in crystalluria in a small group of young boys ($n = 37$, 4–10 years old), but not in individuals with adequate levels of vitamin A ($n = 8$)⁵⁵. Furthermore, vitamin A treatment in children with vitamin A deficiency ameliorated urinary risk factors for stones, including reduced calcium and oxalate and increased citrate and glycans levels⁵⁵. In a case-controlled observational study, adult stone formers ($n = 75$) were shown to have lower circulating levels of vitamin A than healthy individuals ($n = 25$)⁵⁶. However, this evidence was not confirmed in another study in adults, in which plasma vitamin A levels were not shown to be lower in calcium-oxalate stone formers than in healthy individuals⁵¹ (Table 1). An important aspect to consider when examining these results is that blood levels of vitamin A might not be a very sensitive measurement of vitamin A deficiency, as this vitamin is stored in the liver and released into the bloodstream only when needed⁵⁷. Overall, evidence of a direct contribution of vitamin A to stone diseases is weak and limited to a few observational studies and preclinical models. Considering that vitamin A is a multifaceted metabolite that has many roles throughout body systems, which are directed by the present vitamers⁴⁹, investigating how these indirect factors contribute to urolithiasis is worthwhile.

Vitamin A has the notable ability to improve intestinal integrity by effectively reducing gut permeability. Indeed, a weakly positive correlation between serum retinol levels and gut barrier integrity has been observed in children^{58,59}. Specifically, children with ≤ 0.25 mg/l serum retinol showed impaired gut barrier function, as determined by the mannitol/lactulose test^{58,59}. Results from in vitro experiments in human intestinal epithelial cell lines showed that all-trans retinoic acid can improve gut integrity as observed through increased transepithelial

electrical resistance, reduced permeability, and increased expression of the tight junction protein ZO-1 (refs. 60,61). In rodent models, dietary retinoids were shown to be involved in the proliferation and differentiation of the intestinal epithelium^{62,63}. Moreover, vitamin A can bolster immune activity and limit the colonization of pathogenic bacteria that damage the intestinal epithelium through interaction with the resident intestinal immune system⁶⁴. Considering that oxalate is absorbed paracellularly in the absence of calcium^{65,66}, reduced intestinal permeability could reduce oxalate uptake and, consequently, the formation of calcium oxalate stones. This hypothesis is further supported by data showing that in obese mice with increased intestinal permeability, gut absorption of oxalate was elevated, independently of dietary intake⁶⁷. Gut barrier integrity is required to prevent the passive translocation of bacterial endotoxins (such as lipopolysaccharides)

Box 1

Vitamin functions potentially related to nephrolithiasis

Systemic

- Vitamin C and E act as non-specific antioxidants that protect host cells from oxidative damage and in turn might prevent potential calcium oxalate crystal attachment and kidney stone formation.
- Vitamin K₂ is needed for local activation of dephosphorylated-uncarboxylated matrix Gla protein (dp-ucMGP), a Gla family protein that sequesters free calcium and prevents soft-tissue calcification.

Liver

- Vitamin B₆ is a cofactor for the alanine-glyoxylate aminotransferase-mediated degradation of glyoxylate to glycine, instead of oxalate.

Intestines

- Vitamin A can improve intestinal integrity, in turn reducing the translocation of dietary oxalate into the systemic circulation and pathogen colonization.
- Vitamin D controls calcium homeostasis by modulating calcium absorption and excretion.

Immune system

- Vitamin A and D act as immune boosters to maintain host immunity and reduce pathogen colonization, which could enhance gut barrier integrity, in turn reducing the absorption of lipopolysaccharide (LPS) and oxalate.

Kidneys

- Vitamin D controls calcium homeostasis by modulating calcium absorption and excretion.

Bone

- Vitamin D controls calcium homeostasis by modulating calcium absorption and excretion.

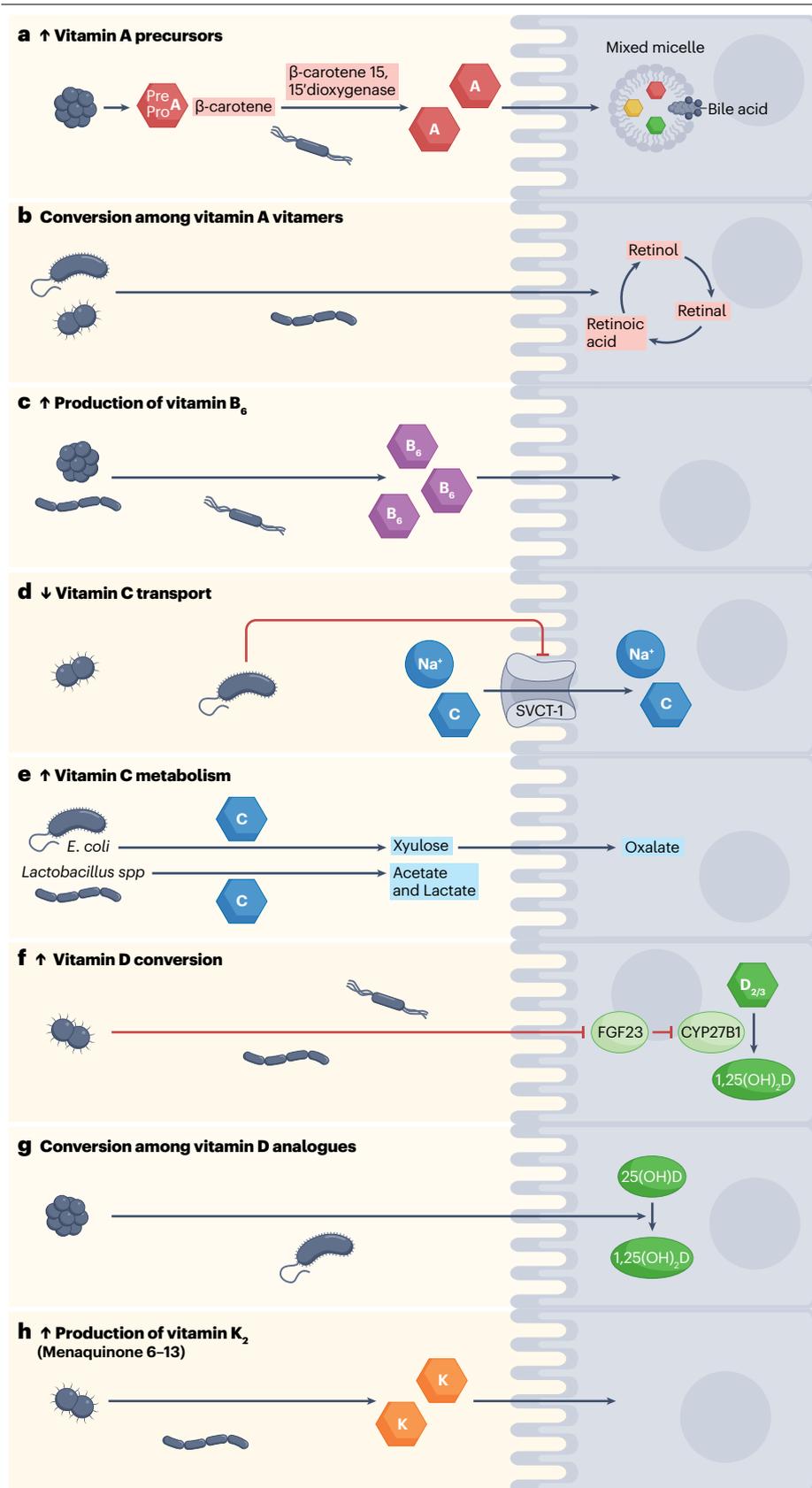


Fig. 1 | Possible roles of the microbiota in host vitamin acquisition and homeostasis. Members of the gut microbiota can **a**, produce vitamin A precursors and can convert β-carotene to retinoic acid⁷¹ and **b**, facilitate the interconversion among vitamin A vitamers^{75,77,78}. **c**, Gut microbiota is also well known to be able to produce sufficient amounts of vitamin B₆ (refs. 47,48,105,106). **d**, Lipopolysaccharide from Gram-negative microorganisms reduces SVCT-1 expression and, in turn, SVCT-1-mediated uptake of vitamin C^{167,170}. **e**, Vitamin C is also metabolized by gut bacteria such as *E. coli*^{159,166} and *Lactobacillus*¹⁵⁶ species to yield xylulose and short-chain fatty acids, respectively; host cells can further process xylulose to yield oxalate. **f**, Through local immune activation, the microbiota can increase the conversion of vitamin D into the active form 1,25(OH)₂D by reducing the expression of FGF23, which normally inhibits CYP27B1-mediated conversion of vitamin D into active vitamin D^{213,214}. **g**, Additionally, multiple members of the microbiota might have the ability to convert vitamin D analogues^{204–209}. **h**, Humans also rely on gut bacteria for the production of vitamin K₂ (refs. 47,303,304,306,307,312,313). SVCT, sodium-dependent vitamin C transporter 1; D_{2/3}, vitamin D₂ and vitamin D₃; 25(OH)D, 25-hydroxyvitamin D; 1,25(OH)₂D, 1,25-dihydroxyvitamin D.

into the bloodstream; in the kidneys, these toxins can induce local damage and further increase stone burden by providing a site for crystal adherence⁶⁸. This hypothesis has been partially supported by the observation that non-infectious calcium oxalate stones contain various levels of endotoxins⁶⁹. These endotoxins could have originated from resident bacteria of the kidney, but gut-derived endotoxins were also shown to be able to reach the kidneys and induce local inflammation⁷⁰.

The interactions between host vitamin A homeostasis and the gut microbiota are quite complex (Table 2). Many bacteria can make carotenoids, but these pigments cannot necessarily be converted into useable retinoids^{71,72}. Exogenous β -carotene, a highly abundant carotenoid found in vegetables, is cleaved to become all-trans retinal by β -carotene 15,15'-dioxygenase⁷³. Most of the bacteria known to express this enzyme are found in marine and environmental locations⁷⁴; however, β -carotene 15,15'-dioxygenase has also been found in human faecal samples, and is predicted, using homologous sequences, to be harboured by *Clostridium* spp., *Streptococcus* spp., *Enterococcus* spp. and *Prevotella marshii*, in which β -carotene 15,15'-dioxygenase might also function to enhance salt tolerance^{75,76}. A notorious food-borne pathogen, *Bacillus cereus*, possesses a multi-functional aldehyde dehydrogenase that is capable of converting both retinoic acid and retinal to retinol⁷⁷. Future determination of gut microbiota members that harbour additional multifunctional enzymes implicated in the conversion of vitamin A vitamers could provide meaningful insights into the role of these bacteria in mediating vitamin A absorption and

function. In addition to these direct systems, the microbiota has an overall influence on retinoid homeostasis, highlighted by an increase in retinoic acid and a decrease in retinol and retinyl palmitate observed in germ-free mice compared with conventionally reared mice⁷⁸. The authors of this study identified a novel mechanism through which bacteria of the Clostridia class can inhibit retinoic acid synthesis in mouse intestinal epithelial cells; additionally, this work highlighted the intimate relationship between the host's micronutrient status and the host's microorganisms⁷⁸. Future work should be aimed at investigating whether commensal bacteria alter the vitamin A profile in stone formers and the potential consequences of these alterations in stone disease.

Vitamin B₆

The generic vitamin B₆ complex encompasses multiple structurally similar water-soluble vitamers: pyridoxal, pyridoxine and pyridoxamine, and the phosphorylated derivatives pyridoxal 5'-phosphate, pyridoxine 5'-phosphate and pyridoxamine 5'-phosphate (Fig. 2). Each phosphorylated vitamer can act as an enzymatic cofactor⁷⁹, but pyridoxal 5'-phosphate is the most biologically active⁸⁰. However, the term vitamin B₆ commonly refers to pyridoxine only. Pyridoxine is a hydroxy methylpyridine with the characteristic methyl group at position 2, a hydroxy group at position 3, and two hydroxymethyl groups at positions 4 and 5 (ref. 81). Mammals cannot produce vitamin B₆, which, therefore, must be obtained through plant-based foods or produced

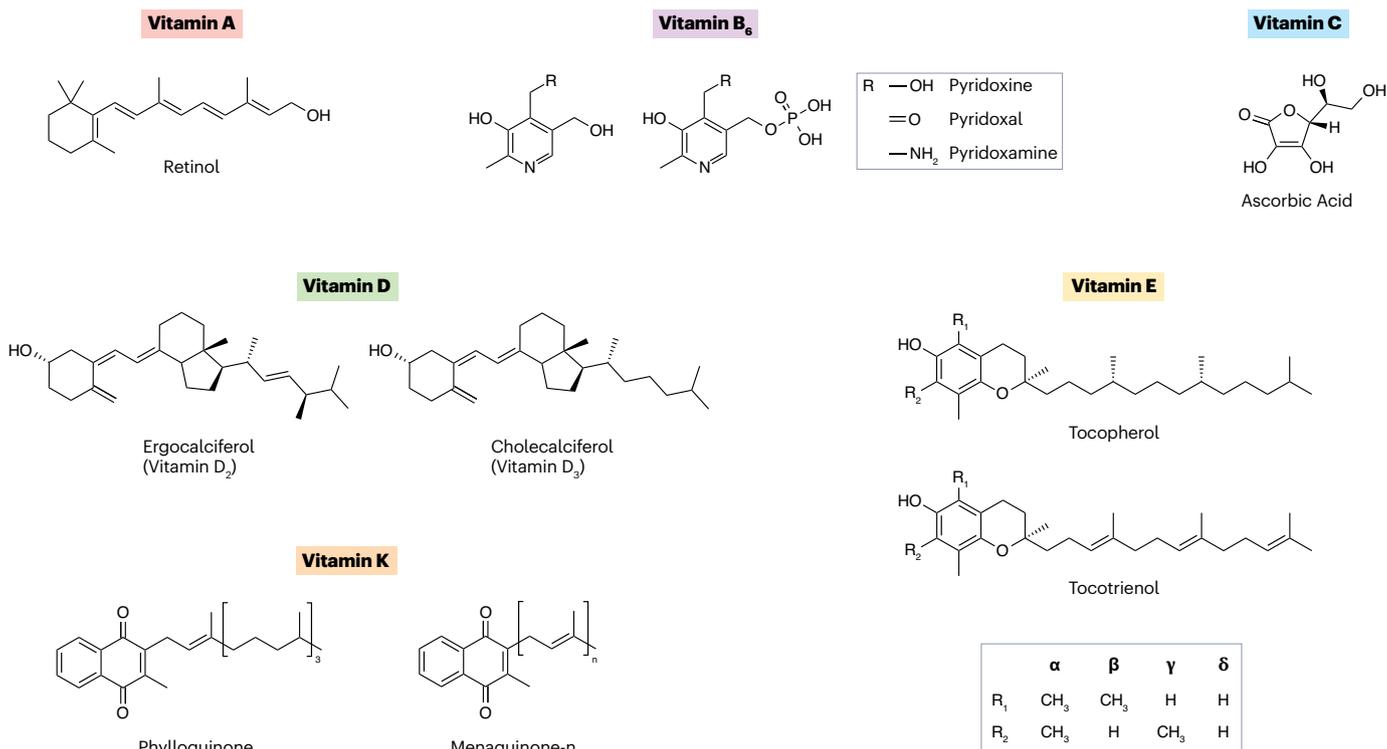


Fig. 2 | Structures of vitamins discussed in this Perspective. Vitamin A (all-trans retinol) contains a β -ionone ring with an isoprenoid side chain and a hydroxyl functional group. Vitamin B₆ is the generic name for six vitamers defined based on the R group and phosphorylation status. Vitamin C (ascorbic acid) weakly resembles a cyclic sugar molecule. Vitamin D₂ (ergocalciferol) and vitamin D₃ (cholecalciferol) structures are defined as secosteroids. Vitamin E contains

four tocopherols and four tocotrienols that are assigned the Greek letters α , β , γ and δ based on the functional group in R₁ and R₂. Vitamin K structures have a characteristic 2-methyl-1,4-naphthoquinone structure and a unique side chain. Vitamin K₁ (phyloquinone) has a phytol side chain and vitamin K₂ (menaquinone) has a polyisoprenyl side chain.

Table 1 | In vivo studies to investigate the role of vitamins in stone disease

Vitamin	Organism	Study group	Main findings	Ref.
Vitamin A	Animal models	Healthy male Wistar rats (young and old)	Vitamin A deficiency resulted in reduced urinary citrate and reduced pH	51
		Healthy female Sprague–Dawley rats	Vitamin A deficiency led to increased renal calculi formation	52
		Male Wistar rats with induced hyperoxaluria (oral 0.5% ethylene glycol)	Rats receiving oral supplementation with retinol had increased urinary pH and citrate but no change in renal accumulation of calcium and oxalate) compared with unsupplemented lithogenic rats	53 ^a
	Human participants	Adult calcium oxalate stone formers and healthy individuals (male and female)	Plasma levels of retinol were not different between sex-matched stone formers and healthy individuals	51
		Male paediatric patients with vitamin A deficiency (Bitot's spots) and healthy individuals	Paediatric patients with vitamin A deficiency had increased urinary calcium and oxalate, reduced citrate and increased presence of crystalluria. In these children, oral treatment with 24,000 IU vitamin A palmitate for 10 days reduced urinary oxalate and calcium to the level of healthy children and increased citrate (but not the level of healthy children)	55
		Adult stone formers and healthy individuals (male and female)	Stone formers had lower plasma vitamin A than healthy individuals (case–control study)	56
Vitamin B ₆	Animal models	Healthy male rats	Rats fed pyridoxine HCl had lower urinary oxalate than untreated rats	89
		Healthy male Wistar rats	Vitamin B ₆ -deficient rats developed calcium oxalate and calcium phosphate crystals in the renal tract	90
	Human participants	Health care workers (adult male and female participants)	Meta-analysis of observational data suggests that vitamin B ₆ intake is not associated with the risk of kidney stones in both males and females (follow-up study)	86
		Health care workers (adult male participants)	Vitamin B ₆ intake is not associated with the risk of kidney stones (prospective study)	87
		Health care workers (adult female participants)	High doses of vitamin B ₆ are associated with a reduced risk of kidney stones (prospective study)	88
		Adults with a history of recurrent renal calculi (male participants)	Oral pyridoxine (250–500 mg/day) for up to 18 months reduced urinary oxalate	93
		Adult recurrent calcium oxalate stone formers (male and female participants)	Oral magnesium oxide (300 mg/day) with pyridoxine-HCl (10 mg/day) for up to 120 days reduced urinary oxalate	94
		Adult patients from a urology stone clinic (male and female participants)	Retrospective dietary intervention and oral pyridoxine (50–100 mg/day) for up to 1 year reduced urinary oxalate and calcium oxalate supersaturation	95
		Adult patients with idiopathic calcium oxalate calculi (unknown gender)	Oral pyridoxine for up to 6 weeks reduced urinary oxalate and glycolate	96
		Adult calcium oxalate stone formers (male and female participants)	Oral pyridoxine (60 mg/day) for 28 days did not change urinary oxalate levels	97
		Adult patients with recurrent idiopathic hypocitraturia (male and female participants)	Oral UriKind-KM6 (1,100 mg potassium citrate, 375 mg magnesium citrate, and 20 mg pyridoxine-HCl) taken every 8 h for 24 h did not change urinary oxalate levels	98
		Adult patients with recurrent idiopathic calcium stone formation and healthy individuals (male participants)	Stone formers had less serum vitamin B ₆ than healthy individuals. Oral pyridoxine (300 mg/day) for 7 days did not change urinary oxalate levels in either group	99
		Adult patients with genetically confirmed primary hyperoxaluria type I (male and female participants)	Oral pyridoxine-HCl (5–20 mg/kg/day) for 24 weeks reduced urinary oxalate	100
Vitamin C	Animal models	Male Wistar rats with induced hyperoxaluria (oral 12% ethylene glycol)	Rats treated with oral vitamin C (100 mg/day) for 2 weeks had no change in renal crystals compared with untreated rats	145
		Male Sprague–Dawley rats with induced hyperoxaluria (oral 2% hydroxyl L-proline)	Rats treated with intravenous vitamin C (500 mg/kg) alone for up to 20 days had reduced calcium oxalate dihydrate crystals in urine, but the formation of other crystal types was unchanged compared with unsupplemented rats	146
	Human participants	Health care workers (adult male participants)	Vitamin C intake is not associated with the risk of kidney stones (prospective study)	87
		Health care workers (adult female participants)	Vitamin C intake is not associated with the risk of kidney stones (prospective study)	88
		Health care workers (adult male and female participants)	Total and supplemental vitamin C intake was associated with an increased risk of kidney stones in men but not in women (observational follow-up study)	122

Table 1 (continued) | In vivo studies to investigate the role of vitamins in stone disease

Vitamin	Organism	Study group	Main findings	Ref.
Vitamin C (continued)	Animal models	Health care workers (adult male participants)	Men who consumed >1,000 mg/day of vitamin C were at a higher risk of stone formation than men who consumed 90 mg/day (observational follow-up study)	123
		Individuals representative of the Swedish male population (adult male participants)	Men who took only vitamin C supplements were more likely to develop kidney stones than men who did not take the supplement (prospective study)	125
		Healthy adults (male and female participants)	Oral treatment with 1 g/day of vitamin C did not change urinary oxalate levels, but 5 g/day and 10 g/day of vitamin C increased urinary oxalate	128
		Adult calcium oxalate stone formers and healthy individuals (male and female participants)	Treatment with oral vitamin C (5 g/day) for 2 days significantly increased urinary oxalate in both groups compared with placebo ($P < 0.01$)	129
		Adult calcium oxalate stone formers and healthy individuals (male and female participants)	Oral vitamin C (2 g/day) significantly increased oxalate absorption and urinary oxalate in stone formers but not in healthy individuals ($P < 0.05$)	130
		Health care workers (adult male and female participants)	Participants who consumed >1 g/day total of vitamin C had excreted a significantly higher amount of urinary oxalate than participants consuming <1 g/day (trend $P < 0.001$)	131
		Adult calcium stone-formers and healthy individuals (male and female participants)	Oral vitamin C (1 g/day and 2 g/day) for 3 days increased urinary oxalate	132
		Adults with sensitivity to acidic foods (male and female participants)	Oral supplementation with vitamin C (500 mg/day) and vitamin C metabolites for 10 days resulted in a lower urinary oxalate than supplementation with vitamin C alone	136
		Adult healthy individuals (male and female participants)	Parenteral vitamin C (100 mg/day and 200 mg/day) increased urinary oxalate in a dose-dependent manner	138
		One adult woman admitted to the hospital with a complex medical history	Treatment with two doses of intravenous vitamin C (unknown concentration) at an alternative medicine clinic led to development of renal calculi (case report)	139
		Two adult men admitted to hospital for SARS-CoV-2	Patients receiving 50 mg/kg vitamin C four times per day developed renal calculi (case reports)	140
		Two adults admitted to the hospital for thermal injuries (one man and one woman)	Patients treated with intravenous vitamin C (66 mg/kg/h) for 18 h and 20 h, respectively, showed calcium oxalate crystals in the kidney (case report)	141
		Adult hospitalized patients receiving a high dose of intravenous vitamin C (male and female participants)	High-dose intravenous vitamin C did not cause renal calculi (prospective study)	142
		Patients with various conditions receiving high-dose intravenous vitamin C	Scoping review of the harms of high-dose intravenous vitamin C. Renal calculi formation was observed in only one case study	143
		Vitamin D	Human participants	Adult kidney stone formers and healthy individuals (male and female participants)
Adult kidney stone formers and healthy individuals (male and female participants)	Long-term vitamin D supplementation resulted in increased risk of hypercalciuria but not increased risk of stone formation compared with placebo (meta-analysis)			189
Health care workers (adult male and female participants)	Vitamin D intake was not associated with kidney stone formation (meta-analysis)			190
Adult patients with a history of calcium kidney stones and individuals with no history of kidney stones from a urology clinic (male and female participants)	Calcium-containing stone formers had lower levels of circulating 25(OH)D than non-stone formers			191
Adult patients with history of calcium kidney stones and individuals with no history of kidney stones (male and female participants)	Serum levels of 25(OH)D were higher in calcium-containing stone formers than in non-stone formers (retrospective study)			192
Adult kidney stone formers and healthy individuals (male and female participants)	Participants with urolithiasis had an increased prevalence of vitamin D deficiency compared with individuals without urolithiasis (prospective case-control study)			193

Table 1 (continued) | In vivo studies to investigate the role of vitamins in stone disease

Vitamin	Organism	Study group	Main findings	Ref.		
Vitamin E	Animal models	Male Sprague–Dawley rats with induced hyperoxaluria (oral 2% hydroxyl L-proline)	Rats treated with intraperitoneal vitamin E (200 mg/kg) alone or in combination with intravenous vitamin C (500 mg/kg) for up to 20 days had reduced calcium oxalate crystals in urine	146		
		Male Sprague–Dawley rats with induced hyperoxaluria (oral 150 mg/day ethylene glycol)	Rats treated with an excess (2,000 U/kg) of vitamin E had fewer renal calculi and reduced oxidative stress markers after receiving ethylene glycol than rats receiving adequate (100 U vitamin E/kg) amounts of vitamin E and vitamin E-deficient rats	230		
		Male Wistar rats with induced hyperoxaluria (oral 0.75% ethylene glycol)	Rats receiving two intraperitoneal injections per week of 200 mg/kg vitamin E for 6 weeks had a reduced Tiselius Risk index for stone oxidative stress markers than unsupplemented rats	231		
		Male Wistar rats receiving lithogenic diet (calculi-inducing diet)	Rats receiving 400 mg/kg of vitamin E had increased production of antioxidant enzymes and reduced markers of lipid peroxidation compared with control rats	232		
		Male Sprague–Dawley rats with induced hyperoxaluria (oral 2% hydroxyl L-proline)	Rats treated with intraperitoneal vitamin E (200 mg/kg) alone or in combination with intravenous vitamin C (500 mg/kg) for up to 20 days had reduced calcium oxalate crystals in urine	146		
	Human participants	Adult calcium oxalate stone formers and healthy individuals (male and female participants)	Plasma levels of tocopherol were higher in sex-matched stone formers than in healthy individuals	51		
		Adult stone formers and healthy individuals (male and female participants)	Stone formers had lower plasma vitamin E than healthy individuals (case-control study)	56		
		Adult patients with lithiasis (male participants)	Stone formers had significantly ($P < 0.05$) lower levels of vitamin E than reference values observed in healthy individuals	233		
		Adult patients with fat malabsorption with or without kidney stones (male and female participants)	Vitamin E levels were lower and markers of oxidative damage were higher in stone formers than in non-stone formers	234		
		Adult calcium oxalate stone formers and healthy individuals (male and female participants)	Stone formers had lower vitamin E and increased markers of oxidative damage than healthy individuals	235		
		Adult stone formers and healthy individuals (male and female participants)	Stone formers receiving vitamin E (400 mg/day) for 9 months produced Tamm–Horsfall protein with an increased inhibitory activity against calcium oxalate crystals compared with stone formers who did not receive vitamin E	238		
		Vitamin K	Animal models	Male Sprague–Dawley rats with induced hyperoxaluria (oral 0.75% ethylene glycol)	Rats treated with vitamin K ₂ for 6 weeks showed reduced crystal deposition compared with untreated rats	290
			Human participants	Paediatric patients with bladder stones (male participants)	Gla residues found in amino acids isolated from calcium oxalate and hydroxyapatite stones	276
Adult patients with kidney stones	Gla residues found in amino acids isolated from calcium oxalate and hydroxyapatite stones			277		
Adult patients with calcium oxalate kidney stones and non-stone formers	Reduced number of Gla residues and lower crystal affinity of a human glycoprotein (nephrocalcin) in stone formers than in non-stone formers			280		
Adults from Flemish population (male and female participants)	Increased levels of plasma dp-ucMGP were associated with an increased risk of stone formation (Mendelian randomization study)			291		
Adult first-time stone formers and healthy individuals (male and female participants)	No change in serum levels of dp-ucMGP in stone formers compared with healthy individuals (prospective study)	292				

HCl, hydrochloric acid; Dp-ucMGP, dephosphorylated-uncarboxylated Matrix Gla protein; Gla, γ -carboxyglutamate. *No statistics performed in the study.

by bacteria^{48,82}. In the body, dietary pyridoxine is converted into pyridoxine 5'-phosphate and pyridoxal 5'-phosphate, and is involved in >140 biochemical reactions in humans⁸³, including a role as a cofactor for the synthesis of amino acids and neurotransmitters. Specifically, vitamin B6 acts as a cofactor for the enzyme alanine-glyoxylate aminotransferase (AGT), which is used in the conversion of glyoxylate to glycine⁸⁴. A deficiency in this vitamin results in the reduction of AGT activity, which might lead to decreased glyoxylate detoxification and, in turn, increased conversion to oxalate, similar to what happens

in primary hyperoxaluria type 1 (PH1, which results from mutations in *AGXT*). Increasing the amount of oxalate circulating in the body is a known risk factor for the development of calcium oxalate kidney stones⁸⁵. Coherent with this hypothesis, pyridoxine supplementation was shown to be useful in the prevention of kidney stones^{86–88}. However, conflicting results have generated a debate around this topic.

Some of the earliest and most compelling evidence that links vitamin B₆ deficiency to calcium oxalate kidney stone development stems from work in feline and murine models (Table 1). In these studies, animals

with B₆ deficiency had increased urinary oxalate and incidence of kidney stones compared with non-vitamin B₆-deficient animals^{89,90}. Vitamin B deficiency was also associated with marked renal damage in cell line models, which contributed to crystal adherence and growth, and might have resulted from the excessive formation of endogenous oxalate^{85,89–91}. Attempts to replicate these findings in humans have yielded conflicting results that question the role of this vitamin in kidney stone disease⁸⁶. In two large-scale studies, the Health Professional Follow-up Study and the Nurses' Health Study⁹², the health outcomes of >40,000 men and women, respectively, were monitored for 6–14 years^{87,88}. The intake of vitamins and kidney stone incidence were among the parameters included in the investigation. In men, no association between vitamin B₆ consumption and the risk of idiopathic stone formation was observed⁸⁷. However, among women, the consumption of >40 mg/day of vitamin B₆ was associated with a lower incidence of kidney stones (relative risk (RR): 0.75, 95% CI 0.52–1.09) compared with women who consumed <3 mg/day of vitamin B₆ (RR: 1)⁸⁸. Unfortunately, the strength of these findings is questionable, as results from a follow-up study involving the same cohorts after >10 years from these results showed no protective effect of vitamin B₆ against stone disease in either men or women⁸⁶. Vitamin B₆ given to stone formers was shown to reduce urinary oxalate^{93–96} in some studies, but, in other studies, no influence of vitamin B₆ on urinary oxalate was observed^{97–99} (Table 1). These disparities could explain why no conclusion has been reached on the use of vitamin B₆ to mitigate calcium oxalate stone disease; additional studies are needed to determine the effect of vitamin B₆ on urinary oxalate.

Similar to what was observed for oxalate kidney stones, incongruous results regarding the role of vitamin B₆ in primary hyperoxaluria were reported. Oral supplementation of vitamin B₆ was shown to reduce urinary oxalate by ~25% in ~50% of patients with primary hyperoxaluria, but a notable variability in the efficacy was observed¹⁰⁰. This variability was attributed to different AGT mutations – with the best response to treatment observed in patients harbouring the G508A mutation – although the reason for this evidence is not understood. Perhaps, in non-responders, underlying factors limit the conversion of pyridoxine to the active form pyridoxal 5'-phosphate¹⁰⁰. In most studies including patients with either primary hyperoxaluria or calcium oxalate stones, vitamin B₆ was supplemented with the pyridoxine vitamer; however, results from in vitro studies in cell line models suggest that the effect of pyridoxine on AGT function can differ among hosts harbouring different *AGTX* genetic variants¹⁰¹, and that pyridoxal or pyridoxamine vitamers could be more beneficial than pyridoxine in hosts harbouring rare *AGT* variants¹⁰². Together, these data showed that the efficacy of AGT could be mediated by the B₆ vitamer, which is used as a cofactor.

The variability in responsiveness to vitamin B₆ could also be influenced by the microbial enzymes present in the gastrointestinal tract^{99,100,103,104}. The gut microbiome can potentially modulate the bioavailability or vitamers of vitamin B₆ that are absorbed (Table 2). A microbiota transplant from a wild type mouse to mice genetically deficient for *EPHB6* – encoding a receptor tyrosine kinase of the EPH family – has been shown to restore circulating pyridoxal 5'-phosphate levels, which had a reduced abundance in the microbiome of these mice¹⁰⁵. This effect could be ascribed to the evidence that many microorganisms (including *Acinetobacter* spp., most *Bacteroides* spp., *Bifidobacterium* spp., *Klebsiella* spp., *Prevotella* spp., some *Clostridium* spp. and some *Ruminococcus* spp.) have all the enzymes to produce pyridoxine de novo^{48,106}. In other bacteria, enzymatic pathways to produce pyridoxine are incomplete, and metabolite sharing between microorganisms is required to obtain pyridoxine^{48,106}. In total, ~85%

of the human dietary reference intake of vitamin B₆ is predicted to be sourced from the gut microbiota⁴⁸. Preliminary results showed a reduction in the abundance of vitamin B₆-related genes in the gut microbiota of stone formers inferred through 16S rRNA gene sequencing⁴³. Vitamin B₆ vitamers are also important to sustain a healthy gut microbiome. For example, *Faecalibacterium prausnitzii* – a presumed beneficial species that is decreased in stone formers³⁸ – is an auxotroph for pyridoxal but not pyridoxamine, highlighting the importance of vitamin B₆ vitamers in preserving the health of bacterial species^{106,107}.

Results from a cross-sectional study showed a 27% decrease in in serum pyridoxal 5'-phosphate concentration in calcium stone formers compared with healthy individuals ($P < 0.0001$), even after pyridoxine supplementation⁹⁹. This study is single-centred, but highlights the importance of assessing the blood concentration of B₆ vitamers in future works to determine whether vitamers reach the circulation upon supplementation. Results from works on primary hyperoxaluria indicate that B₆ vitamers could have differential consequences for the activity of AGT, and, ultimately, for urinary oxalate^{101,102}. Bacteria typically make pyridoxal, pyridoxine and pyridoxamine, which can be used as reactants but, sometimes, are also the final products of specific pathways in bacterial vitamin B₆ synthesis¹⁰⁸. Thus, ingested or synthesized pyridoxal might be converted through incomplete pathways and metabolite sharing occurring in the “pantryome”⁴⁷ into other vitamers that might be responsible for the different urinary oxalate titres observed across studies. Considering these hypotheses, determining how the composition of the microbiota relates to the circulating vitamer titres in stone formers and non-stone formers would be an interesting area to explore.

Vitamin C

Vitamin C is the common name for L-ascorbic acid and L-dehydroascorbic acid, the oxidized form of ascorbic acid. These vitamins are water soluble and have a structure that weakly resembles a cyclic sugar molecule (Fig. 2). Differently from most animals, humans lack a functional gluconolactone oxidase, the enzyme required for the biosynthesis of ascorbic acid, and, therefore, are unable to produce this vitamin de novo¹⁰⁹. The main sources of dietary vitamin C are fruits and vegetables, including tomatoes, potatoes, bell peppers and citrus fruits. Dietary vitamin C is readily absorbed in the intestine through sodium-dependent vitamin C transporters (SVCT1 and SVCT2)¹¹⁰. However, vitamin C is a water-soluble vitamin and, therefore, only limited amounts of vitamin C are stored in the body, whereas the majority of this vitamin is eliminated through urinary excretion¹¹¹. For this reason, ascorbic acid must be consumed regularly to effectively counter the rapid excretion of vitamin C and maintain sufficient levels in the body¹¹². Owing to the rapid excretion of vitamin C, dietary intake of vitamin C is better captured by blood and urine measurements of ascorbic acid, whereas vitamin C intracellular content would provide a realistic overview of bodily vitamin C status¹¹³. The prevalence of vitamin C deficiency is low in high-income countries and increasing in low-income and middle-income countries¹¹⁴.

In the body, vitamin C acts as a non-specific antioxidant and protein cofactor, and might prevent infection^{115,116}. A combination of genetic investigations and radiolabelling studies indicated that dehydroascorbic acid, 2,3-diketogulonic acid and oxalate are the major metabolites produced from the catabolism of vitamin C in mammals^{111,117}. Thus, increased oxalate in the urine might, in part, depend on vitamin C metabolism, although the role of vitamin C as a risk factor for kidney stone development is strongly debated¹¹⁸ (Table 1).

Table 2 | Studies to assess the role of the gut microbiota in vitamin homeostasis

Vitamin	Main findings	Role of microbiota in vitamin homeostasis	Ref.
All vitamins	All vitamin synthesis pathways are found in the human faecal metagenome	Synthesis of vitamins	46
Vitamin A	Bacteria synthesize many types of carotenoids that might be converted into vitamin A	Source of vitamin A	71
	A gene encoding β -carotene 15,15'-dioxygenase is predicted to be present in the genomes of <i>Clostridium</i> spp., <i>Streptococcus</i> spp., <i>Enterococcus</i> spp. and <i>Prevotella marshallii</i>	Potential conversion among vitamin A vitamers	75
	<i>Bacillus cereus</i> expresses a multi-functional aldehyde dehydrogenase that can convert retinoic acid and retinal into retinol	Conversion among vitamin A vitamers	77
	Bacteria in the Clostridia class can inhibit mouse retinoic acid synthesis	Reduction in circulating vitamin A	78
Vitamin B ₆	Several vitamin B ₆ genes were found to be present in members of the gut microbiota ^a	Potential source of vitamin B ₆	47
	The gut microbiota is predicted to produce over 80% of the recommended dietary intake of vitamin B ₆	Source of vitamin B ₆	48
	Microbiota transplant from wild type to vitamin B ₆ -genetically deficient mice restored circulating levels of vitamin B ₆	Source of vitamin B ₆	105
	Numerous bacteria from the gut microbiota have functional pathways for vitamin B ₆ synthesis	Source of vitamin B ₆	106
Vitamin C	<i>Lactobacillus helveticus</i> CICC 22171 requires vitamin C to grow	Catabolism of vitamin C	155
	<i>Lactocaseibacillus rhamnosus</i> GG metabolizes vitamin C to lactate, acetate and CO ₂	Metabolism of vitamin C into other metabolites	156
	<i>Escherichia coli</i> K-12 can ferment L-ascorbate to D-xylulose 5-phosphate through the <i>ula</i> gene cluster ^a	Metabolism of vitamin C into other metabolites	159
	<i>Streptococcus pneumoniae</i> encodes for part of the <i>ula</i> gene cluster	Catabolism of vitamin C	161
	<i>Salmonella enterica</i> encodes for part of the <i>ula</i> gene cluster	Catabolism of vitamin C	162
	<i>Shigella flexneri</i> encodes for part of the <i>ula</i> gene cluster	Catabolism of vitamin C	163
	<i>Enterococcus faecalis</i> V583 has homologs of the <i>ula</i> gene cluster and can grow on vitamin C	Catabolism of vitamin C	164
	<i>Klebsiella pneumoniae</i> 13882 encodes for part of the <i>ula</i> gene cluster and metabolizes L-ascorbate	Catabolism of vitamin C	165
	The <i>Escherichia coli</i> ascorbate transporter has a high affinity for vitamin C	Catabolism of vitamin C	166
	LPS released by <i>E. coli</i> O111:B4 reduces the expression of SVCT1 and SVCT2 in Caco-2 cells and in mice, reducing the uptake of vitamin C	Reduced absorption of vitamin C	167
	Infection with enteropathogenic <i>E. coli</i> E2348/69 reduces the expression of SVCT1 and SVCT2, increases the expression of SVCT inhibitory miRNA (miR103a, miR141, miR200a) and reduces vitamin C uptake in mice	Reduced absorption of vitamin C	170
Vitamin D	In men, serum 1,25(OH) ₂ D partly explained the variance in α -diversity (within sample diversity) and β -diversity (between sample diversity) and was associated with a change in abundance of 12 taxa	Potential metabolism of vitamin D	202
	Serum 25(OH)D was associated with certain bacterial taxa and thought to mediate vitamin D absorption	Microbiota potentially mediates vitamin D absorption	203
	<i>Streptomyces sclerotialis</i> FERM BP-1370 can convert 25(OH)D into 1,25(OH) ₂ D through cytochrome P-450	Conversion among vitamin D vitamers	204
	The gene encoding <i>Streptomyces griseolus</i> P450SU-1 (CYP105A1) converts vitamin D ₂ and D ₃ to 25(OH)D forms and converts 25(OH)D ₃ to 1,25(OH) ₂ D ₃	Conversion among vitamin D vitamers	205
	<i>Pseudonocardia autotrophica</i> NBRC 12743 converts vitamin D ₃ into 25(OH)D ₃ or 1,25(OH) ₂ D ₃ through <i>vdh</i>	Conversion among vitamin D vitamers	206
	<i>Bacillus lehensis</i> G1 converts 1 α -hydroxyvitamin D ₃ into 1,25(OH) ₂ D ₃ through CYP107CB2	Conversion among vitamin D vitamers	207
	<i>Bacillus lehensis</i> G1 converts vitamin D ₃ to 25(OH)D ₃ through CYP107CB2	Conversion among vitamin D vitamers	208
	<i>Ruminococcus torques</i> encodes for a gene that shows partial homology with human CYP27A1 ^a	Potential conversion among vitamin D vitamers	209
	Germ-free mice have lower titres of circulating vitamin D than conventional mice, and have increased titres of vitamin D after colonization with the pathogen <i>Citrobacter rodentium</i> , which regulates immunity to promote vitamin D absorption	Altered vitamin D absorption	213
	Butyrate (microbiota metabolite) enhances VDR production and binding of 1,25(OH) ₂ D ₃ to VDR	Altered absorption and function of vitamin D	219
	Administration of <i>Limosilactobacillus reuteri</i> NCIMB 30242 increased serum 25(OH)D levels in otherwise healthy adults with hypercholesterolaemia	Increased absorption of vitamin D	223

Table 2 (continued) | Studies to assess the role of the gut microbiota in vitamin homeostasis

Vitamin	Main findings	Role of microbiota in vitamin homeostasis	Ref.
Vitamin D (continued)	Co-administration of <i>Lactocaseibacillus paracasei</i> DG and vitamin D3 increases serum 25(OH)D levels in mice	Increased absorption of vitamin D	225
Vitamin E	α -tocopherol quinone and α -tocopherol quinol are found in many types of bacterial cells	Potential source of vitamin E	240
	Mice receiving antibiotics in combination with vitamin E had increased titres of vitamin E compared with mice only receiving vitamin E	Altered absorption of vitamin E	244
	Chickens receiving antibiotics in combination with vitamin E acetate had increased titres of vitamin E compared with chickens only receiving vitamin E acetate	Altered absorption of vitamin E	245
	Mice monocolonized with <i>Lactobacillus acidophilus</i> NCFM had reduced titres of α -tocopherol acetate in the small intestine compared with germ-free controls	Altered absorption of vitamin E	251
	Certain members of the gut microbiota express a bile salt hydrolase that can deconjugate bile acids and potentially alter the absorption of fat-soluble vitamins	Potentially altered absorption of vitamin E	248
Vitamin K	Several members of the gut microbiota express vitamin K ₂ genes ^a	Potential source of vitamin K ₂	47
	<i>Veillonella</i> spp., <i>Bacteroides</i> spp. and <i>Eubacterium lentum</i> produce menaquinones with different side chain length	Source of vitamin K ₂	303
	<i>Prevotella</i> spp. produce menaquinones with different side chain length	Sources of vitamin K ₂	304
	Members of the gut microbiota have incomplete pathways for menaquinone synthesis	Potential source of vitamin K ₂	306
	Members of the gut microbiota have incomplete pathways for menaquinone use	Potential metabolism of vitamin K ₂	307
	Increased abundance of specific members of the gut microbiota associated with faecal vitamers of vitamin K ₂	Potential source of vitamin K ₂	308
	Increased abundance of specific members of the gut microbiota associated with faecal vitamers of vitamin K ₂	Potential source of vitamin K ₂	309
	<i>Bacteroides</i> spp. were higher in postmenopausal women with high blood vitamin K ₂ than women with low blood vitamin K ₂	Potential source of vitamin K ₂	310
	The diversity of the gut microbiota of patients with Crohn's disease correlated negatively with vitamin K deficiency (as measured by inactive osteocalcin)	Potential source of vitamin K ₂	311
	Healthy participants taking antibiotics had lower circulating menaquinones than participants who did not take antibiotics (measured post-mortem)	Potential source of vitamin K ₂	312
Mice given antibiotics had reduced caecal vitamin K ₂ compared with controls	Potential source of vitamin K ₂	313	

LPS, lipopolysaccharide; VDR, vitamin D receptor. ^aReview article.

Canadian Urological Association clinical guidelines continue to recommend limiting vitamin C intake to <1,000 mg/day to theoretically prevent hyperoxaluria, whereas limiting vitamin C intake is not mentioned in the American Urology Association or European Association of Urology guidelines for stone disease^{119–121}. However, evidence for the role of vitamin C in stone disease is conflicting. In one of the earliest studies dating back to the 1990s – the Health Professionals Follow-up Study – no difference in the relative risk of stone formation was reported in men who consumed $\geq 1,000$ mg/day of vitamin C (from foods and supplements) compared with men who consumed <250 mg/day of vitamin C⁸⁷. These findings were confirmed after 2 years in women, in the Nurses' Health Study⁸⁸. Conversely, in a follow-up study in which the same cohorts were monitored >10 years after these studies, both total and supplemental vitamin C intake was significantly associated with an increased risk of kidney stones in men ($P = 0.005$), but not in women ($P = 0.01$)^{122,123}. Importantly, in these follow-up studies, a baseline of 90 mg/day, instead of the previously used 250 mg/day, was used to reflect changes in the Recommended Dietary Allowance (minimum dietary intake to meet nutritional requirement) of vitamin C; thus, the statistically significant results reported in the follow-up studies were probably partially ascribable to the definition of adequate vitamin C intake¹²⁴. A report on the Cohort of Swedish Men, which is a population of >45,000 men aged 45–79 years recruited in 1997 and

undergoing follow-up monitoring in 2008/2009 and 2019, showed that men who specifically took vitamin C supplements, but not multivitamins containing vitamin C, were nearly twice as likely to develop stones than men who did not take the supplements¹²⁵. These long-term studies rely on the voluntary recording of dietary and supplement intake; thus, several confounding factors – the most obvious of which is dietary misreporting – could contribute to the inconsistencies observed across these studies¹²⁶. Regardless of these discrepancies, evidence hints at the possibility that the role of ascorbic acid in stone disease could be sex specific. Evidence from a review¹²⁷ in which vitamin C data from multiple studies were summarized showed that women typically had higher blood levels of vitamin C than men. The mechanisms underlying this difference have not been determined, but the reduction in circulating vitamin C in men could be hypothesized to be the result of increased metabolism to oxalate; however, additional data are required to support this speculation.

In a study in which the effect of vitamin C on urinary oxalate was investigated¹²⁸, marginal increases in urinary oxalate were observed after treatment with up to 10 g/day of ascorbic acid in healthy individuals. In other studies, small but consistent increases in urinary oxalate were also shown upon supplementation with >1,000 mg/day of vitamin C in both healthy individuals and patients with kidney stones^{129–132}. Interestingly, calcium oxalate stone formers seemed to have larger

increases in urinary oxalate upon oral administration of ascorbic acid than non-stone formers, suggesting that individual patient factors are likely to contribute to stone disease^{129,130,132}. Whether oral ascorbic acid is converted into oxalate in the gastrointestinal tract before absorption is unclear, but results from studies in which the absorption of ¹³C₂-oxalate was investigated showed that calcium oxalate stone formers absorbed more oxalate than healthy individuals, suggesting that increased oxalate absorption could be a driving factor for calcium oxalate stone disease^{133,134}.

Caution should be taken when interpreting the results from these studies because the non-enzymatic conversion of urinary vitamin C into oxalate can occur rapidly and during the analysis phase^{128,135}. However, results from a small-scale crossover study in adults with sensitivity to acidic foods showed that the intake of vitamin C in combination with downstream metabolites (excluding oxalate) resulted in a smaller increase in urinary oxalate than vitamin C alone, suggesting that the observed increases in urinary oxalate are likely to be enzymatic in nature¹³⁶. The rate at which vitamin C is converted into oxalate can be hypothesized to be variable among individuals, but oral administration is associated with confounding variables, such as the gut microbiota, which can metabolize vitamin C, making it difficult to infer meaningful conclusions about the fate of supplemented vitamin C. Intravenous administration of ascorbic acid bypasses the gastrointestinal tract and directly enters the bloodstream, providing a more accurate representation of how vitamin C reacts in circulation than that obtained with oral administration. Both high and low doses of intravenous ascorbic acid increase urinary oxalate^{137,138}, and the formation of renal calculi with intravenous vitamin C has been documented in multiple case studies^{139–141}. However, in a large prospective study, no renal stones were observed in patients receiving intravenous vitamin C, also in patients with a history of stones¹⁴². This evidence is corroborated by a scoping review¹⁴³, in which the review of 73 studies showed that intravenous vitamin C did not result in the development of renal calculi. In only one case report analysed in this scoping review¹⁴³ one patient developed renal calculi after intravenous treatment of vitamin C at an alternative medicine clinic¹³⁹. The majority of evidence shows that intravenous vitamin C increases urinary oxalate, but does not necessarily result in the formation of renal calculi. However, results from studies in which oral consumption of vitamin C was used point towards some indication that vitamin C can increase the risk of renal calculi, although this evidence is not strong. The rationale behind a stronger support towards an involvement in stone formation for vitamin C received orally rather than intravenously is difficult to determine. Perhaps the route of consumption could be the missing component in understanding how vitamin C could directly contribute to stone disease, but additional research is needed to fill in this gap.

An overlooked aspect of vitamin C with regard to stone disease is the unique ability of this vitamin to act as a water-soluble, non-specific antioxidant. This evidence first emerged from a study in which vitamin C alone or in combination with vitamin E (α -tocopherol) was shown to ameliorate ROS generation and oxalate-induced markers of oxidative damage in renal tubule cell lines¹⁴⁴. However, the combination approach was the most effective at decreasing the toxicity of calcium oxalate crystals¹⁴⁴. These claims have been refuted through in vitro experiments with Madin–Darby canine kidney cells and in vivo experiments using male Wistar rats in which no effect of vitamin C as an effective antioxidant to reduce calcium-oxalate-associated ROS generation was shown¹⁴⁵. However, in another study in which a Sprague–Dawley rat model was used¹⁴⁶, vitamin C alone was shown to be able reduce the

abundance of calcium oxalate dihydrate crystals at some time points, and of some – but not all – markers of oxidative damage; however, the abundance of calcium oxalate monohydrate crystals was unchanged¹⁴⁶. The contrasting outcomes among these two studies^{145,146} could be explained by important differences in the experimental approach used. For example, Wistar rats were used in one study¹⁴⁵, and received oral vitamin C treatment along with ethylene glycol administered in the drinking water; conversely, male Sprague–Dawley rats were used in the other study¹⁴⁶, and were treated with intravenous vitamin C, whereas hydroxyl L-proline was added to the drinking water. In addition to the metabolic shifts caused by ethylene glycol and hydroxyl L-proline¹⁴⁷, Sprague–Dawley rats might be more susceptible to kidney stones than Wistar rats¹⁴⁸. Thus, additional experimental evidence is needed to determine whether vitamin C can mitigate oxidative damage from kidney stones and whether these effects are enhanced by combination therapy with vitamin E.

The ability of oral vitamin C to be converted into oxalate yields the potential to exacerbate stone disease, but this observation is inconsistent among individuals. Thus, other factors that mediate vitamin C homeostasis in humans must exist in the gastrointestinal tract (Table 2). The gut microbiome can synthesize vitamin C, but the amount of vitamin C synthesized through this route is minimal compared with that coming from dietary sources^{149–151}. However, oral consumption of vitamin C can potentially alter the microbiota because of the ability of specific members of the microbiota to metabolize ascorbic acid^{152–154}. Some lactobacilli can convert vitamin C^{155,156} into acetate and lactate, which are non-toxic metabolites that increase microbiome function through bioenergetic pathways^{47,157} and might promote the colonization of these oxalate-degrading bacteria¹⁵⁸. Moreover, using the *ula* (utilization of L-ascorbic acid) gene cluster¹⁵⁹, bacteria such as *Escherichia coli* can metabolize ascorbic acid to D-xylulose, which can be absorbed and further broken down into oxalate¹⁶⁰. This same *ula* gene cluster is also found in pathogenic bacteria such as *Streptococcus pneumoniae*¹⁶¹, *Salmonella* spp.¹⁶², *Shigella flexneri*¹⁶³, *Enterococcus faecalis*¹⁶⁴ and *Klebsiella pneumoniae*¹⁶⁵. The *E. coli* ascorbic acid transporter also shows a higher affinity for ascorbic acid than the mammalian SVCT1, suggesting that the bacteria can compete with the host for vitamin C¹⁶⁶. Additionally, *E. coli* can reduce host uptake of ascorbic acid through the release of lipopolysaccharides (LPS)¹⁶⁷, which increases NF- κ B-dependent production of TNF, which in turn reduces the expression of sodium-dependent vitamin C transporters SVCT1 and SVCT2 through the inhibition of *SLC23A1* and *SLC23A2* promoters (encoding SVCT1 and SVCT2, respectively)^{168,169}. Pathogenic *E. coli* also increases the expression of multiple host microRNAs that directly block the production of SVCT1 and SVCT2 (refs. 170–172). Taken together, these results show that the composition of the microbiota seems to potentially have a role in the metabolism of vitamin C before this vitamin is even absorbed by the host. The microbiota of stone formers is more commonly enriched in pathogenic bacteria that typically harbour the *ula* gene cluster compared with the microbiota of non-stone formers, and these individuals showed a larger increase in oxalate levels when receiving oral vitamin C than non-stone formers; thus, whether the microbiota of these individuals is converting oral vitamin C into a metabolite that is readily absorbed and easily converted into oxalate would be interesting to postulate. Moreover, the extent to which commensal bacteria influence vitamin C absorption and subsequent stone formation is unknown, and is an important knowledge gap in this area, as these microorganisms constitute a large proportion of the microbiota.

Vitamin D

Vitamin D is the generic identifier that refers to a group of fat-soluble secosteroids with crucial biological properties. In humans, vitamin D is primarily found as naturally synthesized vitamin D₂ (ergocalciferol) or vitamin D₃ (cholecalciferol), but other forms also exist (Fig. 2). For example, vitamin D₄ (22-dihydroergocalciferol), another naturally derived form of vitamin D, is present in some fungi. Synthetic versions like vitamin D₅ (1 α -hydroxy-24-ethyl-cholecalciferol) have also been produced and tested as antitumour therapeutics. Generally, vitamin D is obtained from diet, supplements and ultraviolet light exposure from the sun. Oily fish, mushrooms, egg yolks and fortified milk are the best dietary sources for vitamin D₂ and D₃ (ref. 173). Natural synthesis of vitamin D₃ requires epidermal exposure to ultraviolet B radiation; however, the efficiency of this production relies on the time and intensity of sunlight exposure, and on skin pigmentation¹⁷⁴. Regardless of the synthesis method, both vitamin D₂ and D₃ are transported throughout the body through blood circulation to be processed for local use (Fig. 3). The vitamins go to the liver for hydroxylation to the storage form, 25-hydroxyvitamin D (shortened to 25(OH)D)¹⁷³. In the kidneys, 25(OH)D is converted to the active form calcitriol (1,25(OH)₂D), which can travel to effector locations to perform different functions¹⁷³. Both 25(OH)D and 1,25(OH)₂D can be actively degraded to maintain the necessary titres of vitamin D¹⁷³.

In humans, the active form of vitamin D regulates multiple bodily processes, but is mostly known for a role in modulating the absorption, excretion and storage of calcium. To facilitate active transcellular absorption of calcium, 1,25(OH)₂D binds the vitamin D receptor (VDR) in the intestinal epithelium, stimulating the uptake of dietary calcium in epithelial cells, where calcium can bind calcium-binding proteins^{175,176}. These proteins transport calcium to the basolateral side of the epithelium, from which calcium is actively pumped into the bloodstream^{175,176}. Calcium can also be absorbed paracellularly, but this phenomenon is dependent on the strength of the calcium gradient, and usually occurs in a vitamin D-deficient state^{175,176}. Conversely, vitamin D has minor roles in influencing calcium excretion from the kidneys¹⁷⁷. The majority (~70%) of calcium reabsorption in the kidney occurs paracellularly in the proximal tubule^{178,179}. The next most common area of reabsorption is the distal tubule, in which calcium is reabsorbed by vitamin D-mediated transcellular transport^{178,180}. The process of distal tubule reabsorption is regulated by 1,25(OH)₂D-mediated increased expression of apical calcium receptor proteins (transient receptor potential cation channel subfamily V member 5 (TRPV5)¹⁸¹ and TRPV6 (ref. 182)), intracellular calcium-binding proteins (calbindin-D_{28K}^{181,183}), and basolateral calcium transporters (Na⁺/Ca²⁺ exchanger 1 (NCX1) and plasma membrane Ca²⁺ ATPase 1b (PMCA1b)¹⁸³). Together, these proteins facilitate distal tubule calcium reabsorption¹⁷⁹. Additionally, vitamin D participates in a feedback loop with parathyroid hormone (PTH) to influence control over renal calcium reabsorption¹⁷⁹. Briefly, low serum calcium triggers the production of PTH, which increases the production of 1,25(OH)₂D in the kidney and, in turn, increases renal calcium reabsorption¹⁷⁹.

Vitamin D modulates the dietary absorption and renal reabsorption of calcium, and also acts on bone cells to regulate calcium homeostasis and bone integrity. Bone metabolism is tightly controlled by osteoblasts (bone-forming cells) and osteoclasts (bone-absorbing cells), which are regulated by vitamin D signalling through VDR. Mice lacking functional VDR showed reduced serum calcium, which resulted in reduced bone mineral density compared with heterozygous VDR mice, highlighting the importance of vitamin D in calcium absorption

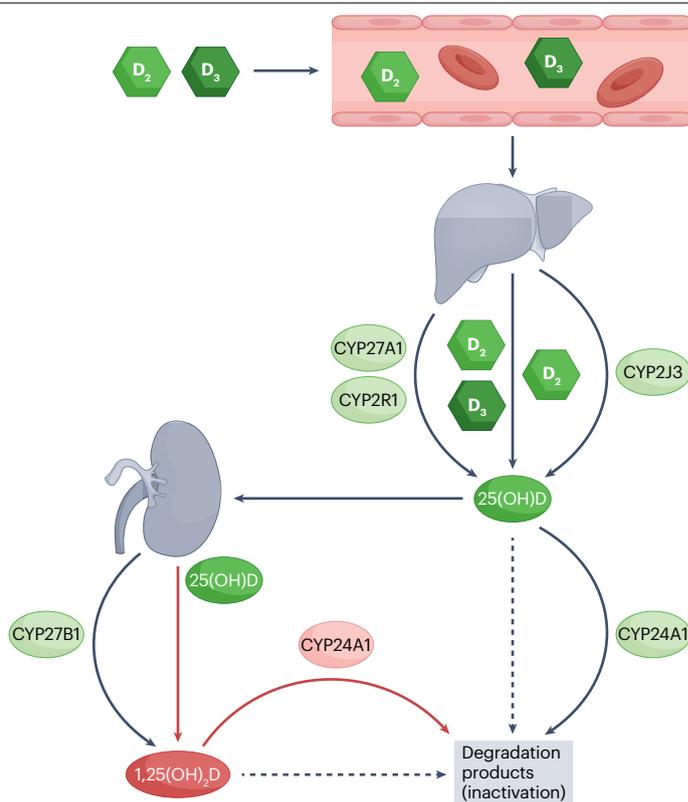


Fig. 3 | Vitamin D metabolic pathway. Vitamin D₂ and D₃ – synthesized by the skin or acquired from ingestion – are circulated to the liver, where the hydroxylation of these vitamins by CYP27A1, CYP2R1 or CYP2J3 occurs. Vitamin D₂ and D₃ are converted into ergocalciferol (25-hydroxyergocalciferol) and calciferol (25-hydroxycholecalciferol), respectively. Together, these products are referred to as 25-hydroxyvitamin D (shortened to 25(OH)D). In the proximal convoluted tubules of the kidneys and in some immune cells (not shown), 25(OH)D is converted into the active form calcitriol (also known as 1,25 dihydroxyvitamin D (1,25(OH)₂D)), through hydroxylation by CYP27B1. Both 25(OH)D and 1,25(OH)₂D can be degraded by CYP24A1 to maintain the necessary titres of vitamin D. CYP, cytochrome P450.

and bone integrity¹⁸⁴. VDR can also act directly on bone cells, as mice harbouring an osteoblast-specific knockout of *Vdr* showed increased bone mass compared with mice with functional *Vdr*¹⁸⁵. Active vitamin D also stimulates the differentiation of osteoclasts¹⁸⁶, in turn promoting normal bone resorption¹⁸⁷.

The relationship between vitamin D-mediated calcium homeostasis and the risk of kidney stone disease is a well-studied topic, but the conclusions are still not clear¹⁷⁹ (Table 1). In a meta-analysis¹⁸⁸ of five studies including stone formers and healthy individuals, calcium-containing stone formers were shown to have increased titres of circulating 1,25(OH)₂D compared with non-stone formers ($P = 0.002$), but no significant difference in 25(OH)D levels was observed between the two groups ($P = 0.56$). One consequence of this increase in 1,25(OH)₂D is that calcium-containing stone formers might be at a substantially increased risk of hypercalcaemia, which could result in hypercalciuria. However, stone-forming individuals with hypercalciuria had increased titres of 25(OH)D but similar 1,25(OH)₂D levels compared with stone formers with normocalciuria and with healthy individuals¹⁸⁸. Thus, only specific

vitamers of vitamin D seem to potentially have a role in hypercalciuria. Results from multiple meta-analyses have shown that vitamin D supplementation increases blood calcium levels but does not enhance the risk of stone disease^{189,190}. Results from one of the meta-analyses¹⁸⁹ in which the effect of long-term supplementation with vitamin D on urolithiasis was investigated concluded that vitamin D supplements do not pose a risk of stone formation ($P = 0.10$)¹⁸⁹. However, consideration should be given to the fact that one of the nine studies included in this meta-analysis accounted for 73% of the weighted decision and that large confidence intervals were reported in the included studies, which could imply that responders and non-responders might exist¹⁸⁹. In summary, vitamin D seems to modulate urinary calcium levels, but might not necessarily contribute to the risk of stone formation, potentially owing to the multifaceted nature of stone disease.

In three studies^{191–193} that were not included in the meta-analysis¹⁸⁸, 25(OH)D levels were slightly decreased in stone formers and a higher proportion of individuals with vitamin D deficiency (as measured by 25(OH)D) was observed among stone formers than among non-stone formers, indicating that vitamin D deficiency could still be prevalent in calcium-containing stone formers. Clinically, hesitancy exists with regard to treating vitamin D deficiency in stone formers because of the preconceived idea that increasing vitamin D will exacerbate stone disease through hypercalciuria, although this hypothesis has been contradicted by experimental evidence^{189,190,194}. However, vitamin D has important roles throughout the body, which might also affect stone formation. The manifestations of vitamin D deficiency include increased inflammation and oxidative damage, which can aggravate stone disease¹⁹⁵. Moreover, vitamin D status (as measured by 25(OH)D) correlates negatively with PTH levels¹⁹⁶; thus, low 25(OH)D could result in increased PTH, which will activate bone resorption and induce hypercalcaemia and subsequent hypercalciuria, potentially increasing the risk of stone formation¹⁹⁷. Results from multiple studies have shown that low bone mineral density is a risk factor for stone disease, but the serum levels of vitamin D were not addressed in these studies^{198,199}. A reduction in vitamin D could also lead to reduced VDR signalling, which is essential for the control of hundreds of genes, including those involved in immunity, the cell cycle, DNA-damage repair, and metabolism^{200,201}. These processes are not explicitly linked to stone disease, but further research could help us to understand whether vitamin D could indirectly influence calcium-containing stone disease through the regulation of these processes.

The apparent inconsistencies regarding the effect of vitamin D on stone formation leave the field open to debate about the relationship between vitamin D and disease aetiology. The majority of work in which the role of 25(OH)D in urinary calcium titres has been investigated produced very conflicting evidence about the function of 25(OH)D in hypercalciuria and in stone disease. Perhaps, the active vitamer 1,25(OH)₂D could provide additional information on this relationship, considering the multifaceted role of this vitamer in human physiology. Thus, in future studies assessing vitamin D status in stone formation, both 25(OH)D and 1,25(OH)₂D should be considered.

The role of the gut microbiota in vitamin D metabolism has not been investigated with regard to stone disease, but has begun to be studied in other fields. Results from a study including octogenarian men showed that circulating levels of active vitamin D (1,25(OH)₂D), but not 25(OH)D, are associated with an increase in alpha diversity of the gut microbiota²⁰². However, in a large genome-wide association study, a correlation between 25(OH)D levels and a higher or lower abundance of specific gut bacterial species was shown; unfortunately,

1,25(OH)₂D was not evaluated in this study²⁰³. The diversity of microbial metabolism within the human intestine and the contribution of partial metabolic pathways to the ‘pantryome’⁴⁷ might partially explain the variations in vitamin D status observed in stone formers. The role of commensal microorganisms in vitamin D metabolism is a relatively unexplored topic, although bacteria might modulate vitamin D homeostasis through direct and indirect mechanisms (Table 2). Specifically, multiple environmental bacteria in the Actinomycetia class possess a cytochrome P-450 (CYP) enzyme capable of converting vitamin D into 25(OH)D (storage form) or 1,25(OH)₂D (active form)^{204–206}. The soil isolate *Bacillus lehensis* G1 was also shown to possess CYP enzymes that can convert vitamin D₃ into 25(OH)D₃, suggesting that this function is present in multiple lineages of bacteria^{207,208}. The genes encoding these unique CYP enzymes have not been found in the genomic pool of human intestinal bacteria; however, a protein having partial homology with human CYP27A1 was identified in *Ruminococcus torques*, which is part of the human gut microbiota²⁰⁹. The potential conversion of vitamin D into 25(OH)D by bacteria in the gut might lead to an increase in circulating vitamin D levels owing to the potentially increased bioavailability of 25(OH)D²¹⁰. Conversely, some bacteria might also reduce the bioavailability of vitamin D. *Mycobacterium tuberculosis*, although not part of the gut microbiota, can infect humans and degrade vitamin D through CYP124 for immune evasion in human phagocytic cells^{211,212}.

Gut microorganisms can also indirectly affect vitamin D activity. In support of this hypothesis, germ-free mice have reduced titres of vitamin D analogues in the circulation owing to increased levels of fibroblast growth factor 23 (FGF23), a protein that inhibits CYP27B1 expression and, therefore, the conversion from 25(OH)D to 1,25(OH)₂D^{213,214}. Upon re-colonization with a defined set of eight commensal microorganisms, these mice experienced a reduction in FGF23 levels and a subsequent increase in circulating vitamin D²¹³. Additionally, colonization of these mice with an enteric pathogen resulted in excessive inflammation and reduction of vitamin D levels until the inflammation passed, indicating that infection-induced inflammation can also regulate vitamin D status²¹³. This evidence is corroborated by other works in which circulating vitamin D was shown to be inversely correlated with TNF levels^{215,216}, a marker of inflammation that acts through the NF- κ B signalling pathway to decrease the expression of colonic vitamin D receptors²¹⁷. These observations support the hypothesis that members of the microbiota can indirectly induce a vitamin D receptor dysfunction in an attempt to shut off the host immune system, which results in reduced uptake of vitamin D²¹⁸.

Beneficial microorganisms in the gut have the ability to affect the downstream function of vitamin D in the host through the production of metabolites. For example, the short chain fatty acid butyrate, which is produced by beneficial microorganisms in the gut microbiota, increases VDR expression, effectively enhancing the binding of active vitamin D to VDR^{219,220}. Butyrate-induced upregulation of VDR, in combination with sufficient vitamin D levels, can reduce inflammation and combat pathogen insult, which could restore the microbiota of stone formers to a healthy and non-inflammatory status, similar to that of non-stone formers²²¹. In line with these observations, results from a genome-wide association study showed that genetic variants of the human VDR locus can influence the composition of the gut microbiota, potentially through the gut–liver axis and the modulation of bile acid metabolism²²².

Certain bacteria seem to also modulate vitamin D acquisition. In a study in which *Limosilactobacillus reuteri* (formally *Lactobacillus reuteri*) NCIMB 30242 was given to adults with hypercholesterolaemia, increased

blood levels of 25(OH)D were observed²²³. Vitamin D is absorbed through micelles in a similar manner to cholesterol; thus, the authors proposed that the bacterium could alter the bile acid metabolism and in turn affect fat-soluble vitamin absorption, but this hypothesis was not confirmed, and the true mechanism remains unknown^{223,224}. A suggested mechanism for *Limosilactobacillus reuteri*-mediated vitamin D absorption is that bacteria can solubilize vitamin D through the production of biosurfactants. *Lacticaseibacillus paracasei* DG (formally *Lactobacillus paracasei*) and *Lacticaseibacillus rhamnosus* GG (formally *Lactobacillus rhamnosus*) were shown to create a stable suspension of vitamin D₃ in water²²⁵. *L. paracasei* DG co-administered with vitamin D₃ to mice daily for 1 week resulted in a higher titres of 25(OH)D than that observed in control mice receiving vitamin D₃ alone, indicating that the bacterium might improve vitamin D absorption²²⁵. These results have not been confirmed in humans, but the microbiota contains many important enzymes that could mediate the absorption of vitamin D and other fat-soluble compounds²²⁶. Results from these studies, although not yet confirmed in humans, also suggest that inflammation-associated microorganisms might reduce vitamin D absorption, that short-chain fatty-acid-producing bacteria might improve vitamin D absorption, and that specific bacteria can convert between vitamers, which, together, point towards a potential role of the gut microbiota in modulating vitamin D homeostasis. Considering the increased abundance of inflammation-associated bacteria and the reduction of short-chain fatty-acid-producing bacteria in stone formers^{38,45}, determining how the gut microbiota in stone formers could influence vitamin D homeostasis and subsequent stone formation is worthy of investigation.

Vitamin E

Vitamin E is an umbrella term for a group of four tocopherols and four tocotrienols; tocopherols have a saturated (phytyl) side chain, whereas tocotrienols have an unsaturated (farnesyl) chain²²⁷ (Fig. 2). Different methylation patterns on the remaining sites of the chromanol double-ring result in different vitamers of these chemicals, denoted by the Greek letters α , β , γ or δ ; α -tocopherol is the most biologically active form²²⁸. The majority of vitamin E is derived from foods or supplements. Vitamin E contains different vitamer profiles, but nuts, seeds, cooking oils, fish and animal meat account for the largest contribution of dietary vitamin E intake in humans²²⁷.

Vitamin E functions as a fat-soluble antioxidant that scavenges free radicals and limits the toxic effects of these radicals, many of which are involved in stone formation²²⁹. Rats exposed to various calcium oxalate stone-promoting diets and concurrently receiving exogenous vitamin E showed a reduction in crystal deposition^{146,230,231}. Conversely, an increased degree of calcium oxalate crystal binding was observed in the kidneys of rats receiving a diet deficient in dietary vitamin E²³⁰. Exogenous vitamin E is presumed to contribute to the reduction of oxidative stress that occurs with stone deposition. This hypothesis has been supported experimentally in studies in which lithogenic rats fed vitamin E had reduced lipid peroxidation and oxidative stress compared with rats not receiving vitamin E^{146,230}. Rats supplemented with vitamin E also showed an increased activity of enzymes with antioxidant abilities (such as superoxide dismutase, catalase and glutathione reductase)^{230,232}. From these preclinical models, vitamin E can be speculated to detoxify a broad range of ROS and ultimately reduce the necessity for enzymatic detoxification, in turn preserving the active forms and increasing the perceived activity of antioxidant enzymes.

Reduced concentrations of plasma vitamin E (as measured by tocopherol) is commonly observed in the blood of human stone

formers^{56,233–235} (Table 1). However, in one study, plasma concentration of tocopherol was found to be increased in stone formers⁵¹. In this study, the highest levels of vitamin E compared with other studies were reported in stone formers (16.4 mg/l) and the second highest in non-stone formers (12.0 mg/l)⁵¹. The detection method in this study⁵¹ was similar to that used in other studies^{233,234}; thus, the reason why such relatively high values were reported is difficult to ascertain, and the existence of a population-based effect on blood levels of vitamin E might be hypothesized. Similar to what is observed in rats, human stone formers often have increased levels of markers of oxidative stress and a reduction in enzymes involved in oxidative protection^{235,236}. Oxidative stress damages the epithelial tissue, and might also impair urinary proteins, such as the Tamm–Horsfall protein, which protects against calcium-based stone formation²³⁷. Tamm–Horsfall protein isolated from individuals with hyperoxaluria shows compromised ability to inhibit calcium oxalate crystallization during in vitro assays compared with the protein isolated from age-matched and sex-matched healthy individuals²³⁸. Tamm–Horsfall protein isolated from patients in the hyperoxaluria group receiving antioxidant vitamin E supplements partially recovered function compared with that isolated from participants with hyperoxaluria receiving placebo²³⁸. Notably, different post-translational modifications were observed in the Tamm–Horsfall protein between patients with hyperoxaluria receiving vitamin E orally and patients with hyperoxaluria receiving placebo²³⁸. The most substantial change reported in the Tamm–Horsfall protein isolated from patients with hyperoxaluria receiving vitamin E was a reduction in the sialic acid content, which is known to promote calcium oxalate aggregation in vitro, indicating a reduced ability to inhibit calcium-based stone formation in these patients²³⁹. Oxidative stress seems to modulate the post-translational modification of the Tamm–Horsfall protein and reduce the efficacy of this protein to inhibit stone formation. Thus, the ability of vitamin E to detoxify ROS, in addition to reducing epithelial damage, could also preserve the integrity of renal inhibitor proteins.

The interactions between vitamin E and the gut microbiota are less characterized than interactions reported for other vitamins, although vitamin E derivatives (α -tocopherol quinone and α -tocopherol quinol) are present in many bacterial cells²⁴⁰ (Table 2). To date, the biosynthesis of vitamin E has only been characterized in plants and cyanobacteria²⁴¹. However, through bioengineering of microorganisms, vitamin E synthesis has been extended to *Saccharomyces cerevisiae* and *E. coli*^{242,243}. Indigenous bacteria of the gut microbiota have not been observed to directly synthesize vitamin E for host use, but these bacteria might have a role in modulating vitamin E absorption. In support of this idea, mice receiving antibiotics together with vitamin E had higher serum vitamin E titres than mice receiving vitamin E alone²⁴⁴. This effect was only observed when mice were supplemented with vitamin E before sacrifice. Chickens treated with antibiotics showed increased absorption of α -tocopherol acetate, but this result was not recapitulated with γ -tocopherol, suggesting that different mechanisms of absorption based on the conjugates of the vitamer exist²⁴⁵. Taking these findings together, microbiota seems to have an effect on vitamin E bioavailability, but additional work is needed to confirm these observations.

The mechanism of vitamin E absorption is not completely elucidated, but is thought to be similar to the mechanism observed for other dietary lipids, according to which the vitamin is emulsified and incorporated into mixed micelles made of phospholipids that are conjugated to bile acids before passive diffusion or receptor-mediated endocytosis²⁴⁶. Both unconjugated and conjugated bile acids can form micelles, although conjugated micelles are more soluble than

unconjugated ones²⁴⁷. Some intestinal commensals, such as bacteria in the *Bacteroides*, *Clostridium*, *Bifidobacterium* and *Listeria* genera, in addition to the Lactobacillaceae family, possess a bile salt hydrolase that deconjugates bile acids²⁴⁸. In the deconjugated form, bile acids have poor solubility and do not typically participate in micelle formation and, therefore, do not solubilize fat-soluble nutrients effectively, ultimately promoting malabsorption of these nutrients^{247,249,250}. Fat-soluble vitamin absorption mediated by bile acid solubility could explain how vitamin E is absorbed; however, mice monocolonized with *Lactobacillus acidophilus* NCFM, a human intestinal isolate and probiotic strain that encodes for bile salt hydrolase and is unable to degrade α -tocopherol acetate in vitro, had reduced α -tocopherol acetate intestine titres compared with germ-free controls²⁵¹. The authors concluded that *L. acidophilus* NCFM mice had increased absorption of α -tocopherol acetate despite the presence of a bile salt hydrogenase, but these results were not confirmed for hepatic or blood vitamin E titres²⁵¹. Mice receiving *L. acidophilus* NCFM had an altered bile acid profile, marked by an increase in the unconjugated bile acid cholic acid, which the authors proposed to activate pancreatic bile acid-dependent carboxyl ester lipase to mediate the absorption of vitamin E acetate^{251,252}. Unfortunately, the levels of deconjugated bile salts such as deoxycholate and lithocholate were not determined²⁵¹. These findings highlight the unclear nature of fat-soluble vitamin absorption, but the deconjugation of bile acids by bacteria could impair the absorption of other fat-soluble vitamins in addition to vitamin E. This topic should be of general interest because most of the other vitamins, including vitamins A, D and K, are also fat soluble. Considering the increased rates of metabolic disease²⁵³ in stone formers – which can result in vitamin E malabsorption²⁵⁴ – and the crucial roles of vitamin E as a potential antioxidant in mitigating stone formation, it would be interesting to investigate the potential role of gut microbiota in these metabolic conditions and in reducing the absorption of crucial fat-soluble vitamins such as vitamin E.

Vitamin K

Vitamin K refers to a group of lipid-soluble vitamins with a characteristic 2-methyl-1,4-naphthoquinone structure (Fig. 2). Specifically, three main vitamers of vitamin K exist and differ based on the side chain structure, function and location in the human body²⁵⁵. Vitamin K₁ (phyloquinone) is a plant-derived K vitamin that is sourced from leafy green vegetables. This vitamin exists as a single structural variant typically found in the liver, and participates in the coagulation response²⁵⁶. Vitamin K₂ (menaquinone), which can be produced by both bacteria and mammals, exists as multiple structural analogues based on the number of isoprenoid side chain units²⁵⁶. These side chains can exist in 1 to 15 repeats (named MK-1–MK-15), although most research to date has focused on MK-4 and MK-7 (ref. 256). Interestingly, vitamin K₂ variants with long chains have increased bioavailability and half-life compared with short-chain variants, which potentially increases the efficacy of these variants in reducing aberrant soft-tissue calcification^{257–259}. Vitamin K₂ is similar to vitamin K₁ in terms of liver function, but can also circulate throughout the body and have additional roles in health. The third vitamer, vitamin K₃ (menadione), lacks a side chain group and is synthetically produced²⁵⁶. Menadione is rapidly converted into menaquinone in the liver²⁶⁰.

Vitamins of vitamin K function throughout the body to reduce soft-tissue calcification through the activation of carboxylglutamate (Gla) proteins, osteocalcin and Matrix Gla protein (MGP). These proteins are commonly localized to bone cells and are stimulated by the

active form of vitamin D^{261–263}. After production, these proteins are post-translationally modified by γ -glutamyl carboxylase, which activates the protein by converting glutamate residues to Gla residues and, in turn, increases the overall negative charge enabling the protein to interact with calcium²⁶⁴. Vitamins K₁ and K₂ can both be used as co-factors for γ -glutamyl carboxylase, whereas vitamin K₃ shows no activity in this reaction²⁶⁵. However, the presence of vitamin K₁ is restricted to the liver, and sufficient concentrations of local vitamin K are required for Gla protein activation; thus, vitamin K₂ is most likely to be responsible for mitigating aberrant calcification.

MGP is a unique member of the Gla family, which is expressed systemically²⁶⁶, differently from osteocalcin, which is only expressed by osteoblasts²⁶⁷. This unique expression pattern suggests that MGP might directly modulate soft-tissue calcification. Specifically, increased titres of dephosphorylated-uncarboxylated MGP (dp-ucMGP), the inactive form of this protein, have been associated with increased levels of vascular calcification in individuals with chronic kidney disease^{268–270} and type 2 diabetes²⁷¹. A positive correlation between the amount of bodily dp-ucMGP and aortic stiffness has also been observed in individuals considered healthy²⁷². Calcium-containing stone formers have increased degrees of aortic calcification compared with non-stone formers^{273,274}; thus, MGP status could have a role in mediating calcium-containing stone formation.

Nephrocalcin and prothrombin are other Gla family proteins that might have roles in stone disease. Nephrocalcin is typically found in the kidneys and has four isoforms that differ by Gla content: form A has three Gla residues; forms B and C have two Gla residues; and form D does not have any Gla residues²⁵⁶. Prothrombin is a clotting protein that is mainly produced by the liver but is also expressed in the kidneys²⁷⁵. Similar to MGP, both nephrocalcin and prothrombin require vitamin K for carboxylation and subsequently sequestration of calcium ions²⁵⁶. The idea that Gla proteins could be implicated in calcium-containing stone disease came from the observation of Gla residues in amino acids extracted from renal stones composed of calcium oxalate and hydroxyapatite, but not from struvite stones^{276,277}. Results from in vitro studies suggest that nephrocalcin and prothrombin²⁷⁸ inhibit calcium oxalate crystallization, and that the inhibitory effect of higher γ -carboxylation states of nephrocalcin on stone formation is greater than the inhibitory effect of lower γ -carboxylation states of nephrocalcin²⁷⁹. In one report, calcium oxalate stone formers were shown to produce urinary nephrocalcin with fewer Gla residues and lower crystal affinity than non-stone formers²⁸⁰. Nephrocalcin isolated from calcium oxalate stones had comparable amounts of Gla residues with urinary nephrocalcin²⁷⁹, suggesting that nephrocalcin participates in stone formation and few Gla residues could lead to stone formation²⁸¹. With the exception of this study, human trials to assess the role of γ -carboxylation states of nephrocalcin and prothrombin in stone disease are scarce, and this area of research holds great potential.

Considering the role of vitamin K₂ in the local γ -carboxylation, understanding the vitamin K₂ status in stone formers would be interesting. Vitamin K is required for MGP activation; thus, circulating titres of dp-ucMGP have been proposed as a novel marker of vitamin K status²⁸². Circulating titres of vitamin K₂ might be more appropriate than dp-ucMGP to differentiate between K₁ and K₂ (ref. 283). To date, no long-term trials to assess dietary vitamin K₂ intake – similar to studies carried out with vitamins B₆, C and D – are available, partly because evidence of an association between vitamin K₂ and stone disease is still preliminary, and also because dietary intake of this vitamin is restricted to supplements and certain fermented foods.

However, similar to what is observed with other dietary trials, these long-term diet studies might not be the best course of action to understand how menaquinones influence stone burden owing to the difficulty in accurately assessing menaquinone intake. Interestingly, some indication for the role of vitamin K deficiency in stone formation comes from the observation that stone formers have many of the comorbidities that are associated with high dp-ucMGP, such as renal dysfunction²⁸⁴, chronic kidney disease^{268–270}, hypertension²⁸⁵, obesity²⁸⁶ and diabetes²⁷¹. High dp-ucMGP depends on vitamin K status; thus, these comorbidities might suggest that vitamin K deficiency is common in stone formers. Renal cells exposed to calcium oxalate have increased MGP expression^{287,288}, which can reduce the formation of calcium oxalate²⁸⁹, indicating that MGP could inhibit calcium-containing stone formation. Male Sprague–Dawley rats treated with vitamin K₁ (which can be converted to MK-4) showed reduced crystal formation after 6 weeks of supplementation²⁹⁰. To date, the relationship between circulating dp-ucMGP (which presumably derives from vitamin K deficiency) and nephrolithiasis in humans has been assessed in only two studies. In a Mendelian randomization study²⁹¹, increased levels of circulating plasma dp-ucMGP were associated with an increased risk of stone disease ($P < 0.05$). In a prospective study including first-time kidney stone formers and matched non-stone formers²⁹², no association between serum dp-ucMGP and stone incidence or recurrence was observed ($P = 0.66$). The reasons for the different outcomes of these studies are difficult to ascertain, and multicentred studies are needed to confirm the validity of the findings. However, these results highlight that the relationship between dp-ucMGP and stone disease is probably more complex than initially thought, especially considering the fact that the activation of dp-ucMGP is also influenced by genetic variants^{291,293–295}. Perhaps the quantification of urinary dp-ucMGP in combination with blood analysis could clarify the status of dp-ucMGP in the local renal environment and the relationship of dp-ucMGP status with kidney stone formation. Considering that the composition of Randall's plaques is highly similar to that of vascular calcifications, the role of vitamin K status and Gla protein modification in renal calculi development warrants further investigation.

In other fields of study, oral supplementation with menaquinones seems to reduce circulating dp-ucMGP^{296–299}, and reduces the degree of vascular calcification^{297,300,301}, but the effects of these supplements on kidney stones are unknown. In most of these studies MK-7 was used, which has a larger number of isoprenoid side chain units than MK-4, another commonly used menaquinone. MK-7 has been shown to have increased half-life and enhanced bioavailability^{257,259} at the potential cost of reduced efficiency compared with MK-4 (ref. 265). Additional work is needed to determine whether vitamin K₂ supplements will reduce the risk of calcium-containing stone formation, but particular interest could be focused on the ability of large menaquinones to mitigate stone formation, considering the unique pharmacology of these vitamers.

Mammals can produce only one form of menaquinone, and the process occurs through the conversion of dietary vitamin K₁ into MK-4 mediated by the UbiA prenyltransferase domain-containing protein 1 (UBIAD1)³⁰². However, the gut microbiota is able to produce medium- to long-chain menaquinones (MK-7–MK-13). The *de novo* production of menaquinones occurs in many bacteria including *Bacteroides* (which produce MK-9–MK-11), *Prevotella* (producing MK-11–MK-13) and *Veillonella* (which produce MK-6 and MK-7)^{47,303,304} (Table 2). These bacteria use chorismate as a precursor molecule for the conversion to menaquinone through the classical menaquinone pathway

(Men operon) or the futasoline pathway (Mqn operon)^{305,306}. Other gut bacteria contain incomplete menaquinone synthesis pathways and rely on metabolite sharing from the pantryome to produce vitamin K₂ (refs. 47,306,307). Results from multiple studies have shown that blood profiles of menaquinones are directly altered by the composition of the human gut microbiota^{308–310}. Generally, an increase in the proportion of menaquinone-producing bacteria leads to a rise in the blood content of menaquinones^{308–310}. Moreover, results from an observational study including patients with Crohn's disease³¹¹ showed a negative correlation between vitamin K deficiency (as measured by serum titres of the inactive Gla protein, undercarboxylated-osteocalcin (ucOC)) and alpha diversity of the gut microbiota (as measured by Chao1). This correlation suggests that the microbiota could contribute to a person's vitamin K status. Disruption of the gut microbiota with antibiotics decreases serum and caecal vitamin K₂ levels^{312,313}. Together, results from these studies suggest that the microbiota has a role in producing an adequate supply of menaquinones in humans, which could be used to activate dp-ucMGP and effectively reduce the occurrence of aberrant calcification. In a meta-analysis of studies in which the microbiota of stone formers was assessed³⁴, some species of *Prevotella* were found to be more abundant in the gut microbiota of stone formers than in healthy individuals, whereas other species of the genus were more prevalent in non-stone formers than in stone formers. Considering that members of *Prevotella* can produce menaquinones, species-specific influence from this genus on vitamin K₂ production could be explored. Further consideration of the understanding of menaquinone production in the microbiome of stone formers could help to clarify the role of vitamin K₂ production in the aetiology of stone disease.

Future directions

The current understanding of how vitamins influence calcium-containing kidney stone disease is insufficient. Thus, conflicting observations have polarized the literature and clinical guidelines without any strong justification. Considering that vitamins are multifunctional cofactors that participate in a myriad of processes throughout the body, the idea of understanding the roles of vitamins in stone disease can be discouraging. This picture is further complicated considering the ability of gut microbiome to modulate vitamin titres, absorption and function. However, these important relationships can be deduced through rigorously defined experiments and clinical trials.

Different future directions can be proposed to begin to address the role of vitamins in stone disease. First, properly conducted multicentred studies are needed to address a multitude of potential confounding variables in calcium-containing stone formers compared with healthy individuals. In these trials, participants should be stratified based on features such as diet, stone type and sex to truly discern the differences between stone formers and non-stone formers. Crucial research outcomes from these studies would include quantifying vitamer concentrations of vitamins A, B₆, C, D, E or K, which might have roles in kidney stone disease. In these studies, gut microbiota should also be analysed through a systems-level approach including metagenomic, metabolomic and/or metatranscriptomic analyses, corroborated by mechanistic studies to gather a complete understanding of the biochemical networks harboured within the gut microbiota, with the aim of identifying differences in the gut microbiota of stone formers versus non-stone formers. Additionally, well-designed *in vitro* and *in vivo* studies to examine the effect of vitamer and probiotic supplementation on both the gut microbiota and calcium oxalate stone formation could be used to further delineate the microbiome–vitamin–stone

axis. Together, these approaches would help to describe the causal factors of stone disease, including the influence of vitamins and the gut microbiome. Second, *in vitro* and *in vivo* models should be established based on differential features observed in stone formers compared with healthy individuals to deduce the mechanisms through which these hypothetical alterations would participate in stone formation. Germ-free models should be used to investigate the role of the gut microbiota in stone disease, and the influence of the indigenous microbiota on stone formation in the model organisms should also be assessed. However, results from these studies must be interpreted with caution, as model organisms harbour a microbiome that is vastly different from that of humans and possess a unique physiology that might not be representative of vitamin acquisition and stone formation in humans. Model organisms are not ideal for these reasons, but might still help to provide some insight into how specific microorganisms could contribute to stone formation and facilitate the development of new ideas. Third, mechanistic findings from preclinical studies will need to be confirmed in humans to support or induce changes to clinical guidelines or patient care. Trials to validate findings obtained in preclinical models will need to be multicentred and span a diverse patient cohort taking into account patient sex, diet, stone type, ethnicity and medical comorbidities. In the hypothesis that mechanistic findings would be confirmed in humans, model organisms could be used to develop therapeutics, but patient data would ultimately be needed to drive these discoveries into the clinic.

Conclusions

Some useful information can be found by analysing the incongruencies of the multiple studies in which the role of vitamins in kidney stone disease was investigated (Box 1). Specifically, vitamin A seems to improve urinary parameters that mitigate stone formation and reduce intestinal permeability, which might reduce oxalate absorption. Some evidence that vitamin B₆ can alter metabolism to reduce oxalate production exists, but is debated owing to conflicting results, as vitamin B₆ has been shown to either be able to reduce stone formation or to have no effect on stone formation. Vitamin C might be converted to oxalate more proficiently in men than in women, indicating that a sex-specific effect on oxalate production from vitamin C might exist. Vitamin C has antioxidant roles in both men and women, which could be protective from stone disease; however, conflicting results in preclinical models suggest that vitamin C could be ineffective at preventing stone formation. Vitamin D has historically been recognized for the ability to increase blood calcium levels, although this phenomenon does not directly contribute to stone formation; however, additional work is needed to investigate the role of this vitamin in stone formation beyond calcium homeostasis, as vitamin D has roles in multiple pathways throughout the body, including immunity, cell signalling, DNA repair and metabolism. Vitamin E acts as a non-selective antioxidant that can detoxify free radicals, which can reduce crystal adherence to the renal epithelium and maintain the integrity of endogenous urinary protein inhibitors. Vitamin K₂ is capable of activating dp-ucMGP, which can sequester free calcium and might be able to reduce aberrant calcification systemically. However, considering the multifunctional role of these vitamins in human health, unravelling the individual role in stone disease will be challenging. This Perspective ultimately discusses observations in the literature in an attempt to promote a new direction in a field in which prior research has not been definitive.

Perhaps, the microbiota, which is able to produce, modify and influence the absorption of vitamins (Fig. 1) can be the missing piece

to this puzzle. In this Perspective, the surface of how the microbiota influences vitamin homeostasis in the human host is merely scratched, as many of these pathways are not fully characterized, and exist in other microorganisms (such as fungi or archaea) that inhabit the intestine. The functional capacity of the microbiota should be preserved through careful clinical antimicrobial stewardship and other factors such as diet to minimize any potential collateral damage caused by the indiscriminate depletion of microbial cross-feeding networks from which the host also benefits. An increased understanding of the mechanisms underpinning kidney stone disease and the development of new preventative strategies are needed to provide patients with effective therapeutic options, and to help mitigate the substantial clinical and economic burden of the growing prevalence of urinary stone disease.

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J.A.C., G.A.S. and K.F.A. researched data for the article. All authors contributed substantially to discussion of the content. J.A.C., G.A.S., K.F.A. and J.P.B. wrote the article. All authors reviewed and/or edited the manuscript before submission.

Competing interests

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