Functional Roles of B-Vitamins in the Gut and Gut Microbiome

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Abbreviations: Ace2, angiotensin I converting enzyme (peptidyl-dipeptidase A) 2; **AhR**, aryl hydrocarbon receptor; **DRI**, dietary reference intake; **DSS**, dextran sodium sulfate; **EHEC**, Enterohemorrhagic Escherichia coli; **HGM**, human gut microbiota; ***folC2***, folylpolyglutamate synthase type; **IBD**, inflammatory bowel disease; **IF**, intrinsic factor; **MAIT-cell**, mucosal-associated invariant T-Cell; **5-OE-RU**, 5-(2-oxoethylideneamino)-6-D-ribitylaminouracil; 5-**OP-RU,** 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil; **PLP**, pyridoxal 5'-phosphate; **RYGB**, Roux-en-Y gastric bypass; **SMCT1**, sodium-coupled monocarboxylate transporter 1; **SMVT**, sodium-dependent multivitamin transporter; **TC-R**, transcobalamin receptor; **WT,** wild type.

Abstract

The gut microbiota produce hundreds of bioactive compounds, including B-vitamins, which play significant physiological roles in hosts by supporting the fitness of symbiotic species and suppressing the growth of competitive species. B-vitamins are also essential to the host and certain gut bacterium. Although dietary B-vitamins are mainly absorbed from the small intestine, excess B-vitamins unable to be absorbed in the small intestine are supplied to the distal gut. In addition, B-vitamins are supplied from biosynthesis by distal gut microbiota. B-vitamins in the distal colon may perform many important functions in the body; they act as (1) nutrients for a host and their microbiota, (2) regulators of immune cell activity, (3) mediators of drug efficacy, (4) supporters of survival, or the fitness of certain bacterium, (5) suppressors of colonization by pathogenic bacteria, and (6) modulators of colitis. Insights into basic biophysical principles, including the bioavailability of B-vitamins and their derivatives in the distal gut are still not fully elucidated. Here we briefly review the function of single B-vitamin in the distal gut including their roles in relation to bacteria. The prospect of extending analytical methods to better understand the role of B-vitamins in the gut is also explored.

1 Introduction

Recent studies have highlighted the presence of trillions of microbes in the guts of mammals; these bacteria produce several metabolites that play a significant role in the biological processes within the host.[1–8] Studies of the gut bacterium and specific isolated bacteria, particularly *Bifidobacterium* and *Lactobacillus* species, show that intestinal bacterium produce 7 of the 8 B-vitamins.[9-20] B-vitamins are biosynthetic precursors of universally essential cofactors used in numerous metabolic pathways; they are indispensable to the host and gut microbiota alike. With the exception of niacin (vitamin B3), a mammalian host cannot produce B-vitamins *de novo;* there is a strict dependence on an exogenous supply, including from the diet and the gut microbiota (Figure 1). Although it has been thought that B-vitamins are mainly absorbed from the small intestine, there are many B-vitamin transporters expressed in the colon (Table 1).[21,22] The majority of microbes exist in the large intestine; they can be categorized as B-vitamin-producing bacteria and auxotrophic bacteria. As a result, competition and symbiosis occur between the host and bacteria, as well as among bacteria, especially with respect to the auxotrophic species whose viability is strictly dependent on acquiring one or more B-vitamins. *In silico* analysis showed that 20-30% of the gut microbiota lack the capacity to produce essential B-vitamins.[23] Since B-vitamin production is under the control of dietary substrates, some gut bacterium may influence food choices of the host.[24] Moreover, a host with an imbalanced or unfavorable intestinal microbiome, referred to as ‘dysbiosis,’ might have altered B-vitamin metabolism in their gut.

To date, a search of PubMed (www.ncbi.nlm.nih.gov/pubmed, accessed on February 28, 2020)

using the terms 'B-vitamin (B-vitamin, B1, B2, B3, B5, B6, B7, B9, B12, thiamin, riboflavin, niacin, pantothenic acid, pyridoxine, pyridoxal, pyroxamine, biotin, folate, folic acid, or cobalamin)' and 'gut microbiota (or gut microbe)' returned over 323 relevant published articles of which almost 27% were reviews. Despite there being some excellent recent reviews on this topic, our understanding of the physiological importance of B-vitamins in the distal colon has only been addressed in some reports.[25-28] Prior to development of the concept of 'gut microbiota' there had been numerous studies conducted in this field, including studies about bacteria in the hindgut with the potential for growth and vitamin-producing capacity in the host. The importance of gut bacteria as a source of vitamins has been demonstrated further by the observation that germ-free animals require dietary sources of various vitamins that are not required by conventional animals.[29,30] Rodents can practice coprophagy which may indirectly supply B-vitamins through the small intestine. It is therefore, this behavior only confirms the availability of B-vitamins in the fecal bacterial biomass and leaves open the question of the availability of B-vitamins in the distal gut in the non-coprophagic host.

It is challenging to identify *in vivo* evidence about the functional roles of the B-vitamins in the distal gut from a much larger number of animals, from *C. elegans* to humans. Functional differences could exist between B-vitamins in the proximal gut and distal gut for the host, as well as gut microbiota; however, relatively little is known about functional roles of B-vitamin in distal gut. In this review, we focus on the role gut microbes have on modulating B-vitamin availability and the functional role of B-vitamin in the distal gut.

2 B-Vitamins

2.1 Vitamin B1 (thiamin)

Thiamin can be produced by gut bacteria *in silico* and *in vitro*.[12,31] It is estimated that gut microbial thiamin synthesis supplies approximately 2.3% of the daily human requirement of vitamin B1 from intracellular concentration of vitamin, weight of bacteria, and composition of the human gut microbiota (Table 1).[27] Four enzymes implicated in the biosynthesis pathway of thiamin are overrepresented in enterotype 2, which is one of the human gut microbiota compositional clusters enriched by *Prevotella* (Table2).[32] High-affinity thiamin transporters 1 and 2 which are capable of carrier-mediated absorption of thiamin have been identified in human colonic mucosa and epithelial cell lines. [33–37] However, there are no data about the actual importance of the supply of thiamin at the distal gut from gut microbiota to host.

Thiamin is a biosynthetic precursor of thiamin pyrophosphate which is essential to carbohydrate metabolism and neural function. Recently, Kunisawa et al. reported that vitamin B1 is important for glycolysis-dependent host cells, especially in Peyer's patch cells.[38] In this regard, a dietary vitamin B1 deficiency may affect host immune responses via the regulation of differentiation and proliferation of immune cells that may in turn influence the gut microbiota. The researchers also showed that feeding mice a vitamin B1-deficient diet causes a vitamin B1 deficiency after only a week. This suggests that the gut microbiota of mammalian hosts is capable of synthesizing only a minimal shortfall of thiamin under general conditions. In contrast, *Acetobacter pomorum*, a thiamin-producing bacteria, can rescue the development of axenic flies in the absence of dietary thiamin

(Figure 1, Table 1).[39] Thiamin is also important to a specific gut bacterium, *Bacteroides thetaiotaomicron in vitro* (Figure 1, Table 2).[40] Thiamin biosynthesis and its transport system are critical to the growth of *B. thetaiotaomicron*. These results suggest that thiamin produced in gut microbiota have a specific role in the composition or function of the gut microbiome.

2.2 Vitamin B2 (riboflavin)

Excess dietary riboflavin, as well as riboflavin produced by commensal bacteria, comprise the riboflavin present in the distal gut (Figure 1). In addition to lactic acid bacteria which are well-known for producing riboflavin in the gut, a genomic analysis of 256 species of human gut microbes found more than half (56%) of them conserve a group of genes for *de novo* riboflavin biosynthesis.[11,27] Mice and humans harbor the functional riboflavin transporter 3 in their large intestine (Table 1).[41,42] In our previous study, we observed that the riboflavin supplied from gut microbiota played a pivotal role in the host; the gut microbiota was able to provide compensatory riboflavin in the short-term when a dietary riboflavin deficiency was induced in mice (Figure 2, Table 1).[43] The survival of axenic—but not conventional—*Drosophila* was significantly depressed when their diet lacked riboflavin [44]. Qi B et al. showed the importance of live bacteria being able to provide micronutrients in the gut, such as riboflavin, to *Caenorhabditis elegans* (Table 1). [45] Riboflavin supplementation increases the usability of heat-killed bacteria as food and promotes intestinal protease activity in *C. elegans*, suggesting that commensal bacteria are a source of riboflavin in *C. elegans.*

An effect of riboflavin on the growth of extremely oxygen-sensitive bacteria (e.g., *Faecalibacterium prausnitzii*) as agents of electron transfer has been suggested.[46,47] Interestingly, *F. prausnitzii* do not encode genes involved in riboflavin biosynthesis.[48] Because oxygen stress is the main aggravator of anaerobic gut microbiota such as *F. prausnitzii* and *Roseburia*, the acquisition of riboflavin modifies the composition of gut microbiota. Indeed, a preliminary study showed that

dietary riboflavin supplementation for 14 days increased *F. prausnitzii* and concomitantly reduced *E. coli.* in a small group of adult volunteers.[47]

The pyrimidine compounds 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU) and 5-(2-oxoethylideneamino)-6-D-ribitylaminouracil (5-OE-RU) are highly potent activating ligands of the mucosal-associated invariant T (MAIT)-cell (Tables 1).[49,50] Both 5-OP-RU and 5-OE-RU are generated from the riboflavin precursor 5-amino-6-D-ribitylaminouracil through the riboflavin producing pathway in various microbes. Taken together, riboflavin produced in the gut microbiota has roles in numerous hosts. These roles include functioning as an essential nutrient; or as a modulator of bacterium fitness in the gut microbiota and immune function in host. (Figure 1).

2.3 Vitamin B3 (niacin)

Niacin can be made from tryptophan in mammals as well as from intestinal bacteria [51–53]. Mammalian colonocytes possess a carrier-mediated mechanism for the uptake of niacin [54]. Exogenous expression of the human sodium-coupled monocarboxylate transporter 1 in oocytes can transport both nicotinate and its structural analogs, despite substrate selectivity being low (Table 1).[55] Administration of microcapsules containing niacin, which release their contents at the ileocolonic region, increased serum niacin concentration in a dose-dependent manner in human subjects.[56]

Niacin acts as an agonist of cell-surface receptors, niacin receptor 1, which is also known as the hydroxycarboxylic acid receptor 2 or G-protein-coupled receptor 109A. Although niacin was not considered a crucial ligand of this receptor, it is expressed in colonic epithelium and its physiological roles have been studied extensively.[28,57,58] A niacin deficiency results in intestinal inflammation and diarrhea.[59,60] Niacin also exhibits potent antioxidant and anti-inflammatory properties and acts as a modulator of intestinal barrier function and bacterial endotoxin production.[61–63] Therefore, niacin has a direct impact on gut microbiota. Indeed, both tryptophan and niacin (nicotinic acid and nicotinamide) treatment have been shown to revert the composition of the intestinal microbiota of angiotensin I converting enzyme (peptidyl-dipeptidase A) 2 (Ace2) mutant mice (Table 1) [64]. Furthermore, the intake of a microcapsule of niacin (900 to 3000 mg)—but not nicotinamide (30 to 300 mg)—resulted in a significant increase in the population of *Bacteroidetes* (Figure 1, Table2).[56] Since *Bacteroidetes* are deficient in the enzymes nicotinamidase and nicotinamide

phosphoribosyltransferase, and luminal niacin—but not nicotinamide—facilitates growth of the *Bacteroidetes* species in the gut (Table 1).[53] Collectively, these results suggest that niacin may have a favorable effect on gut microbial composition in humans.

2.4 Vitamin B5 (pantothenic acid)

A genomic analysis of 256 representative organisms of human gut microbiota found that *de novo* synthesis of pantothenic acid is limited in *Bacteroidetes* and *Proteobacteria* genomes.[27] Spearman rank correlations suggest that an increased intake of pantothenic acid was related to an increased relative abundance of *Actinobacteria*, which do not possess the ability to synthesize pantothenate (Table 2).[27,65] In addition, *Lactobacillus spp.*, *Streptococcus spp.*, and *Enterococcus spp.*, members of the pantothenate nonproducing *Firmicutes* phylum, require pantothenic acid for their growth *in vitro* (Figure 1, Table 2).[66–68] These reports suggest that a symbiosis exists in the distal gut between pantothenic acid-consuming bacteria and pantothenic acid-producing bacteria. A study has shown the absorption of pantothenic acid across the intestinal loop by sodium-dependent multivitamin transporters (SMVT, SLC5A6) as well as biotin; however, direct evidence is lacking with respect to the absorption of pantothenic acid across the colon. [69–71] Antibiotic-treated mice reportedly exhibit signs of a pantothenic acid deficiency (Figure 1, Table 1).[72] Open questions remain about whether pantothenic acid in the distal gut could be a nutrient for the host, and if its availability modulates the composition of the gut microbiota and host cell function.

2.5 Vitamin B6 (pyridoxine, pyridoxamine, pyridoxal)

Pyridoxal 5'-phosphate (PLP), a common coenzyme form of vitamin B6, can be synthesized *de novo* via two routes.[27] The majority of *Actinobacteria*, *Bacteroidetes,* and *Proteobacteria* (approximately 50% of the 256 human gut microbiota [HGM] genomes) have at least one *de novo* biosynthetic pathway. Reduced concentrations of vitamin B6 in the colonic content of wild type (WT) mice (0.4 to 0.5 mg/100 g) that have been treated with antibiotics (0.1 to 0.2 mg/100 g) are disruptive to their gut microbiota.[73] The administration of probiotics (*Bacteroides acidifaciens*) in this context partially restored the amount of vitamin B6 in the colonic content of antibiotic-treated mice. Luminal metabolic analysis and shotgun DNA sequence analysis showed that the metabolic pathway of vitamin B6 is dynamically changed in various conditions.[74–76] The relative abundance of *Blautia*, *Coprococcus,* and *Roseburia,* which are lower in patients with schizophrenia compared with control patients, was negatively correlated with vitamin B6 metabolism-related genes (Table 2).[75] When a person resistant to atherosclerosis consumes a cholesterol-enriched diet, microbiome genes for vitamin B6 metabolism increase significantly. [74] Distal gut bacteria samples from lean individuals appear to be more involved in vitamin B6 synthesis than samples from obese people based on metaproteomics analysis (Table 2). [76] The maintenance of vitamin B6 biosynthesis in host cells are of great importance for various homeostatic processes in health and disease, including host immune responses (Figure 1).[77–79] Consistent with these reports, the estimated maximal percentage of the daily reference intake of pyridoxine is the highest (86%) of all the 8 B-vitamins[27] The carrier of vitamin B6 has yet to be determined, although it has been characterized as an energy- and

196 temperature- (but not Na-) dependent transporter of pyridoxine in young adult mouse colonic
197 epithelial cells. [80]

198 Two excellent reports have characterized functions of vitamin B6 in the gut microbiota.
199 Scott, et al. showed that microbes integrate nutritional- and drug-cues in *C. elegans* (Table 1).[81]
200 They found that PLP produced by commensal bacterium acts in concert with ribonucleotide
201 metabolism to facilitate the effects of 5-fluorouracil, a drug used to treat colorectal cancer. Inhibition
202 of bacterial ribonucleotide metabolism drastically antagonizes drug efficacy, while inhibition of
203 deoxyribonucleotide metabolism improves it, an effect also regulated by dietary pyrimidines.[81]
204 Administration of vitamin B6-producing bacteria (*B. acidifaciens*), attenuates the colonization of
205 *Salmonella. typhimurium* and promotes recovery from gut inflammation in antibiotic-treated mice
206 (Table 1).[73] It is conceivable that there is an interactive effect of vitamin synthesis and function in
207 the gut microbiota.

208 Mice with a severe vitamin B6 deficiency protected them against dextran sodium sulfate
209 (DSS)-induced colitis.[82] Another study also investigated the effect of dietary vitamin B6 intake on
210 colonic inflammation in the IL10$^{-/-}$ murine model of IBD.[83] This study is highly suggestive because
211 both moderate vitamin B6 supplementation and mild depletion significantly attenuated the
212 histological and molecular features of colonic inflammation. Accordingly, approximately 30% of
213 patients with inflammatory bowel disease (IBD) show evidence of a vitamin B6 deficiency.[84]
214 Therefore, vitamin B6 bioavailability in the gut has an indirect role on colonic inflammatory diseases
215 which include gut infection with enteropathogens and IBD. The prime cause of this indirect effect of

tryptophan metabolites. Supplementation of tryptophan and its metabolite niacin (nicotinic acid and nicotinamide) can rescue intestinal inflammation in Ace2 knockout mice.[64] Conversion of pyridoxamine and pyridoxine to PLP—requiring flavin-mononucleotide (derived from vitamin B2) as a coenzyme—is an essential cofactor for two key enzymes used in the synthesis of niacin from tryptophan.[85] Moreover, the bacterial metabolites implicated include tryptamine, indole, and indole metabolites (i.e., indole-3-aldehyde, indole-3-acetic acid [from some *Lactobacillus*], indole-3-pyruvic acid, indole-3-acrylic acid [from *Peptostreptococcus*], indole-3-lactic acid [from *Bifidobacterium longum* subspecies], and indole-3-propionic acid), or the host metabolite kynurenine, that activates the aryl hydrocarbon receptor (AhR) as the physiological agonist.[86–91] Activation of the AhR pathway may ameliorate DSS-induced colitis in mice.[92] DSS-induced colitis was more severe in AhR knockout mice than in WT mice.[93] Dietary supplementation with 0.5% tryptophan reduced the severity of DSS-induced colitis and ameliorated symptoms in WT mice but not in AhR knockout mice.[94] The production and supply of tryptophan metabolites are complexly regulated by the dietary supply of tryptophan or other nutrients, and the composition of the gut microbiota, such that vitamin B6, B2 and/or B3 availability in the colon may play an important role in the production of tryptophan metabolites.

2.6 Vitamin B7 (biotin)

A genomic analysis of 256 representative organisms of human gut microbiota found that 40% were capable of *de novo* synthesis of the vitamin B7.[27] Studies have shown that the phylum *Bacteroidetes* (48/51 strains), *Fusobacteria* (13/14 strains), and *Proteobacteria* (29/38 strains) predicts the synthesis of biotin.[27,95] In contrast, even though *Actinobacteria* genomes lack an essential biotin biosynthesis gene, 19 of 23 (83%) of them contained a biotin transporter, indicating the need for biotin.[27] This result suggests the potential to control *Actinobacteria*-related diseases via regulating biotin availability in the gut.[96] Another study showed that four enzymes in the biotin biosynthesis pathway are overexpressed in *Bacteroides* enterotype (Table 2).[32] Absorption of biotin in both the small and large intestine occurs via a carrier-mediated process that involves the SMVT system encoded by the *SLC5A6* gene.[70,71,97–100] The colonic absorption rate of biotin measured by [14-C] biotin or the *in vivo* intestinal loop showed that post-ileal biotin absorption was 8 to 12% as efficient as the absorption of biotin after oral dosing in pig.[101,102] Lipopolysaccharides inhibit colonic biotin uptake via interference with membrane expression of its transporter.[103] On the other hand, Hayashi et al. revealed that competition for biotin utilization exists between the host and bacteria (Figure 1, Table 1).[104] *Lactobacillus murinus* consumes and reduces available biotin in the gut and antibiotic-induced dysbiosis promotes alopecia in mice fed a biotin-deficient diet (Table 1 and 2).[104]

2.7 Vitamin B9 (folate)

The microflora of the gut, particularly *Bacteroides*, *Bifidobacteria*, *Streptococcus*, and *Lactococcus* spp. can synthesize folate as a common food fermentation product of carbohydrates during the growth.[15,105–109] A genomic analysis of 256 representative organisms of human gut microbiota estimated that 43% of microbes conserve *de novo* folate synthesis pathway genes [27]. Folate biosynthesized by bacteria can be absorbed by folate transporters in the rat, pig, and human colon.[110–113] Although there is no clear evidence at this time, two types of folate transporters are considered to work in colon, the human proton-coupled folate transporter (SLC46A1) and the reduced folate carrier (RFC; SLC19a1).[114–121] The capacity of the large intestine to absorb forms of folate , equivalent to at least 37%, 18%, or nearly 100% of the daily human need, was estimated via *in silico*, pig, and human studies, respectively.[27,111,113] Folate biosynthesis in gut bacteria and/or transport expression in host are affected by a low-carbohydrate diet, increased protein content, probiotic dietary factors that affect gut microbes (e.g., dietary folate, fiber, oligosaccharide, and/or drug), and prebiotic bacteria supplementation (e.g., *Bifidobacteria*). Based on these influential factors, the composition of gut microbiota and dietary intake interactively modulate the availability of folate from the colon to host (Table 2).[105,106,116,122,123]

Of note, colonic folate also has a role despite that it does not affect plasma folate levels.[124] Virk B, et al. showed that modulating folate uptake, or the folate cycle in the gut bacteria via *E. coli*, but not in the host (*C. elegans*), affects the lifespan of host. *E. coli* mutants influence the lifespan of worms independently of *E. coli* growth. Metformin retards aging in *C. elegans* by altering folate and

methionine metabolism in *E. coli* (Table 1).[125] As observed in the fly, dysbiosis and the consequent overabundance of a specific bacterial group which does not produce folate, may be an important factor in aging (Table 1).[126] These results suggest that bacterial folate synthesis influences the lifespan of hosts by acting on microbial physiology without compromising the host's folate status. The targeted mutation of the bifunctional dihydrofolate synthase/folylpolyglutamate synthase type 2 (*folC2*) gene, essential to the folate synthesis pathway, reduced immunomodulatory histamine production and the anti-inflammatory effect of *L. reuteri* 6475 in a mouse model of acute colitis (Table 1).[127] Similarly, a microbial *folC* mutation reduced intestinal folate production, and increased mRNA expression levels of the folate receptor, RFC, in human colonoids.[128] Cancer cells use folate for growth, so the availability of folate in the distal gut has been associated with the proliferation of colorectal cancers.[129–133] Luminal folate, or folate-derived metabolites, have a role in the regulation of immune function.[28,134] The folate-related metabolite 6-formylpterin antagonizes MAIT cell effector function.[134]

2.8 Vitamin B12 (Cobalamin)

The cobalamin biosynthetic pathway is present in 42% (110/256) of the HGM genomes and can be found in all *Fusobacteria*.[27] In contrast, it is rare in *Actinobacteria* and *Proteobacteria* and half of the *Bacteroidetes* genomes are missing this biosynthetic pathway. A HGM study showed that 83% of bacteria (260/313 species) encode cobalamin-dependent enzymes.[135] Intriguingly, most of these 260 species lack the genes required to synthesize cobalamin. [135] In another report, 75.9% (410/540) of the bacteria were cobalamin-utilizing organisms and only half of those (209/410) possessed the cobalamin biosynthetic pathway.[136] These genome-based analyses indicate that those bacteria rely on cobalamin-uptake mechanisms to acquire sufficient levels of it from the surrounding environment. For example, *Bacteroides thetaiotaomicro*n does not encode genes involved in the cobalamin biosynthetic pathway, but rather it has three homologs of the cobalamin transporter, btuB1, btu2B, and btuB3 and a cobalamin binding factor, BtuG2 (Figure 1, Table 1).[135]

Recently, three cobalamin binding proteins (IF, haptocorrin, and transcobalamin) have been shown to mediate cellular cobalamin up-take in adult mammals via three receptors (i.e., IF-cbl R/cubilin, asialoglycoprotein R, and transcobalamin receptor [TC-R]). Cobalamin transport systems have been reviewed in detail elsewhere.[137–140] However, cobalamin represents only a small portion (1.4%) of the total amount in human feces.[141] Therefore, it is doubtful whether colonic-derived cobalamin has a role in nutrition of the host.

In addition to cobalamin, bacteria can produce a variety of corrinoid derivatives.[142–144] In the presence of many corrinoids, 7 cobalamin analogs were identified and quantitated in human feces

306 (Figure 1, Table 1) [141]. As if to have the ability to respond to it, human gut microbes likely encode at
307 least 27 distinct corrinoid transporter families. [144,145] Supplementation of 3.94 μg/ml
308 cyanocobalamin increased fecal cobalamin and reduced corrinoid analogs concomitant with a lower
309 abundance of *Bacteroide*s in mice (Table 2).[146] These results suggest that competition and exchanges
310 of cobalamin and its analogs could potentially determine microbial fitness. [143,144,147] The functions of
311 corrinoids have been studied in several reports and were thoroughly reviewed by Degnan, et
312 al.[145,148,149] Cobalamin and its derivatives determine not only microbial fitness, but also microbial
313 activity, including pathogenicity in the host. The bacterial transcription factor EutR requires ethanol
314 amine, a precursor of cobalamin, and the cobalamin derivative adenosylcobalamin for the
315 transcription of virulence factors needed for infection of the host and dissemination of
316 enterohemorrhagic *E. coli* (EHEC) serotype O157:H7 and *Salmonella*.[150–153] Cobalamin uptake by
317 the gut commensal bacteria *B. thetaiotaomicron* limits the production of Shiga-Toxin by EHEC [154].
318 Cobalamin also acts as an immunomodulator to promote cellular immunity.[28,155,156] These results
319 indicated that luminal cobalamin and the availability of its analog can modulate the luminal infection.
320

3 Analytical methods

The use of novel and sophisticated methodology will be key to uncovering all the functional roles of B-vitamins and their metabolites in distal colon (Table 3). For example, mass spectrometry will be a powerful tool to find for novel metabolites derived from B-vitamins, as reported in corrinoid derivatives. Isotopic tracing techniques could assess metabolism and uptake of B-vitamins by bacteria and the host in the distal gut. Developing the experimental condition that prevent indirect supply of B-vitamins and its derivatives from fecal bacterial biomass in coprophagic rodents should be also considered.

4 Conclusions

In the distal colon, B-vitamins derived from food and the microbiota help to nourish the host (Figure 1). The amounts of B-vitamins will be different depending on the composition of the gut microbiota. Within the intestinal microbiota, some bacteria utilize rather than synthesize B-vitamins which indicates that bacteria compete in the gut. Microbes also produce other metabolites from B-vitamins that have significant roles in biological processes in the host or bacteria. Because the gut microbiota varies among individuals, the amounts of each B-vitamin in the distal colon will be different among the host and the populations of bacteria. Further investigations in this field, including interactive effects of multiple B-vitamins are warranted. Potentially other metabolites, derived from B-vitamins, play significant roles in the regulation of luminal health or dysregulation (Figure 1). Gut microbiota may synthesize specific metabolites that help us to sense whenever a nutrient is undersupplied, and related signaling or signs could influence our food choices.

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Author contributions

T.U. wrote the manuscript. T.S., K.M., and A.T. contributed to critical discussions. The manuscript was critically reviewed, revised and given final approval by all co-authors. T.U. is the guarantor of this work.

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Figure Legends

Figure 1. The role gut microbes have on modulating B-vitamin and its derivatives availability and the functional role of those in the distal gut.

B-vitamins are synthesized by intestinal microbiota and supply the host and other microbes. When gut microbes metabolize the B-vitamins they are converted into other compounds which have roles both in host cells and in microbes. Biosynthesized B-vitamins, and their metabolic derivatives, are excreted into the feces where they may be reused by animals that are coprophagic.

Figure 2. Time course of the changes in riboflavin (left) and metabolism (right) in the luminal and host when riboflavin deficient diet ingestion. Luminal metabolic changes rapidly occur than in the host. **Conc,** concentration.

642 Table 1 Importance of B-vitamin in distal gut

Vitamin	**DRI[a]**	**Colon transporter (official symbol)[b]**	**Importance of B vitamin in distal gut**
B1, thiamin	2.3%	THTR1 (SLC19A2) THTR2 (SLC19A3)	• Supplies *D. melanogaster* with nutrients*[39]
B2, riboflavin	2.8%	RFVT3 (SLC52A3)	• Supplies mice with nutrients[43] • Supports survival of *D. melanogaster** and *C. elegans**[44,45] • Supports electron transfer in oxygen sensitive bacteria[46,47] • Metabolites activate MAIT-cells by derived from a riboflavin precursor[49,50]
B3, niacin	27%	SMCT1 (SLC5A8), GPR109A (HCAR2)	• Helps growth of Bacteroidetes[53]
B5, pantothenic acid	0.078%	SMVT (SLC5A6)	• Nutrient supply for microbes[66–68] and mice[72]
B6, pyridoxine, pyridoxamine, pyridoxal	86%	N.D.	• Manipulates the therapeutic potential of 5-FU in C. elegans*[81] • Suppresses colonization of pathogenic bacteria in mice[73] • Modulates colitis experimentally in mice[74–86]
B7, biotin	4.5%	SMVT, (SLC5A6)	• Supplies mice with nutrients[104]
B9, folate	37%	hPCFT (SLC46A1) Rfc1 (SLC19A1)	• Folate metabolism in the gut microbiota modulates host aging and life span[124,125] • Helps the anti-inflammatory function of *Lactobacillus reuteri*[127] • Folate and folate-derived metabolites regulate immune function[28,135]
B12, cobalamin	31%	N.D.	• Provides other functional corrinoids[136,145, 147-149] • Cobalamin and cobalamin derived metabolites regulate microbial fitness and pathogenicity[136,143,147]

643 a) DRI were estimated in Magnúsdóttir S, et al.[27] There is a limitation in this estimation of DRI which did not consider the fact that B-vitamins
644 produced by gut bacteria not only supply the host, but also supply other gut bacteria.

b) Mammalian vitamin transporter in the colon was described. N. D.; not determined.

* Depends on the differences in organ structure between insects and mammals, the results from *D. melanogaster* and *C. elegans* did not apply directly to mammals.

5-FU, 5-fluorouracil; Ace2, angiotensin I converting enzyme (peptidyl-dipeptidase A) 2; DRI, dietary reference intake; HCAR2, hydroxycarboxylic acid receptor 2; hPCFT, human proton-coupled folate transporter; MAIT-cell, mucosal-associated invariant T-Cell; Rfc1, reduced folate carrier; RFVT3, riboflavin transporter; SMCT1, sodium-coupled monocarboxylate transporter 1; SMVT, sodium-dependent multivitamin transporter; THTR1, thiamin transporter1; THTR2, thiamin transporter 2.

652 Table 2 Influence of B-vitamins on gut bacteria

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Vitamin	Relationship between B-vitamins and gut bacteria
B1, thiamin	• Biosynthesis pathway of thiamin were overrepresented in *Prevotella* enterotype[32]. • Critical for the growth of *B. thetaiotaomicron in vitro*[40]
B2, riboflavin	• Dietary riboflavin supplementation increased *F. prausnitzii*[47] • Open questions
B3, niacin	• A microcapsule of niacin (900 to 3000 mg) resulted in a significant increase in the population of *Bacteroidetes*[56] • Nicotinamide supplementation with drink improved the composition of the gut microbiota in Ace2 $^{(-/-)}$ mice[64]
B5, pantothenic acid	• Increased intake of pantothenic acid was related to an increased relative abundance of *Actinobacteria*[65] • *Lactobacillus spp., Streptococcus spp., and Enterococcus spp.,* required pantothenic acid for their growth *in vitro* [66-68]. • Open questions
B6, pyridoxine, pyridoxamine, pyridoxal	• The relative abundance of *Blautia*, *Coprococcus*, and *Roseburia* was negatively correlated with vitamin B6 metabolism-related genes[75]. • Distal gut bacteria samples from lean individuals appear to be more involved in vitamin B6 synthesis concomitant with lower ratio of *Firmicutes/Bacteroides* [76].
B7, biotin	• Biotin biosynthesis pathway are overexpressed in *Bacteroides* enterotype[32]. • Critical for the growth of *Lactobacillus murinus* [104]
B9, folate	• Abundance of *Bifidobacterium* and *Lactobacillus* was positively associated with folate status[105]. • Increased total aerobic bacteria was related to an increased total amount of folate in the intestinal content[106].
B12, cobalamin	• Supplies nutrients for *B. thetaiotaomicron in vitro* [135] • Supplementation of cyanocobalamin increased fecal cobalamin concomitant with a lower abundance of *Bacteroides*[146].

Open questions remain whether indicated B-vitamin availability in the distal gut modulates the composition of the gut microbiota.

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Table 3 Methods that could facilitate the uncovering of novel information in the future

1. Mass spectrometry (MS):
   - MS can distinguish B-vitamins from metabolic derivatives (e.g., cobalamin from other corrinoids).
   - MS will be instrumental in the search for novel, or as yet untargeted, metabolites from which new biomarkers might be identified to elucidate the environment inside the gut.
2. Experimental conditions for animal studies:
   - Coprophagy should be taken into account as it has the potential to alter fecal metabolites and dietary intake of vitamins to confound study results.
   - Housing rodents in metal cages or restrainers can help to avoid this.
3. Isotopic tracing techniques:
   - B-vitamins and their metabolites could be quantitatively profiled using isotopes; B-vitamin transport could be traced to assess their metabolism and uptake by bacteria and the host.
4. Transporters and receptors:
   - The functions of B-vitamin transporters expressed in the large intestine are unclear, particularly in a complex environment such as the mammalian colon.
   - Being able to specifically knockout or inhibit a gene within the large intestine could improve our understanding in relation to functionality.
5. Reconstruction of the condition of the lumen:
   - Complicated processes, such as secretion, can utilize B-vitamins or B-vitamin metabolites synthesized by bacteria.

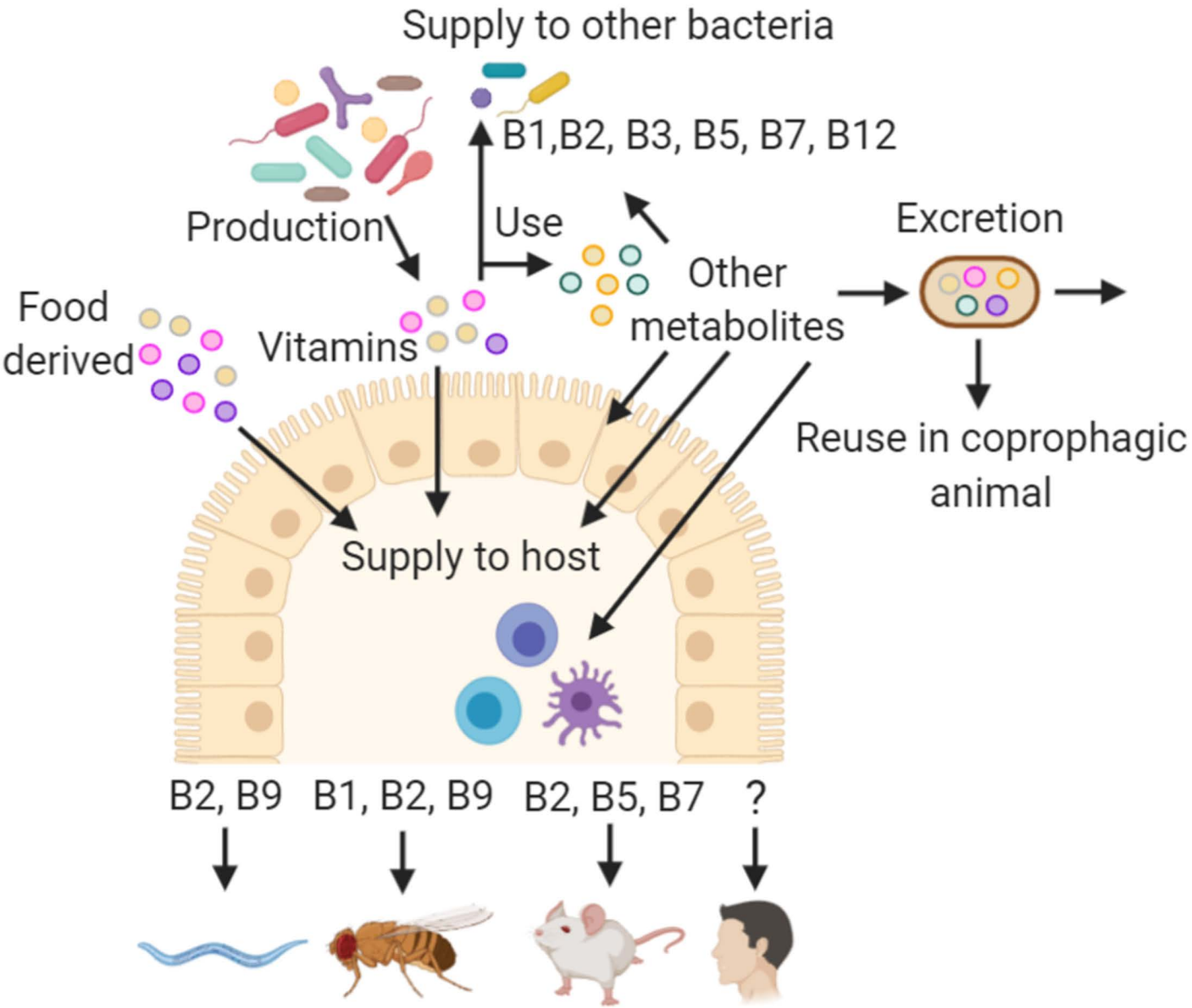
Supply to other bacteria
B1,B2, B3, B5, B7, B12
Production
Use
Excretion
Other
metabolites
Food
derived
Vitamins
Reuse in coprophagic
animal
Supply to host
B2, B9
B1, B2, B9
B2, B5, B7
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Figure 1

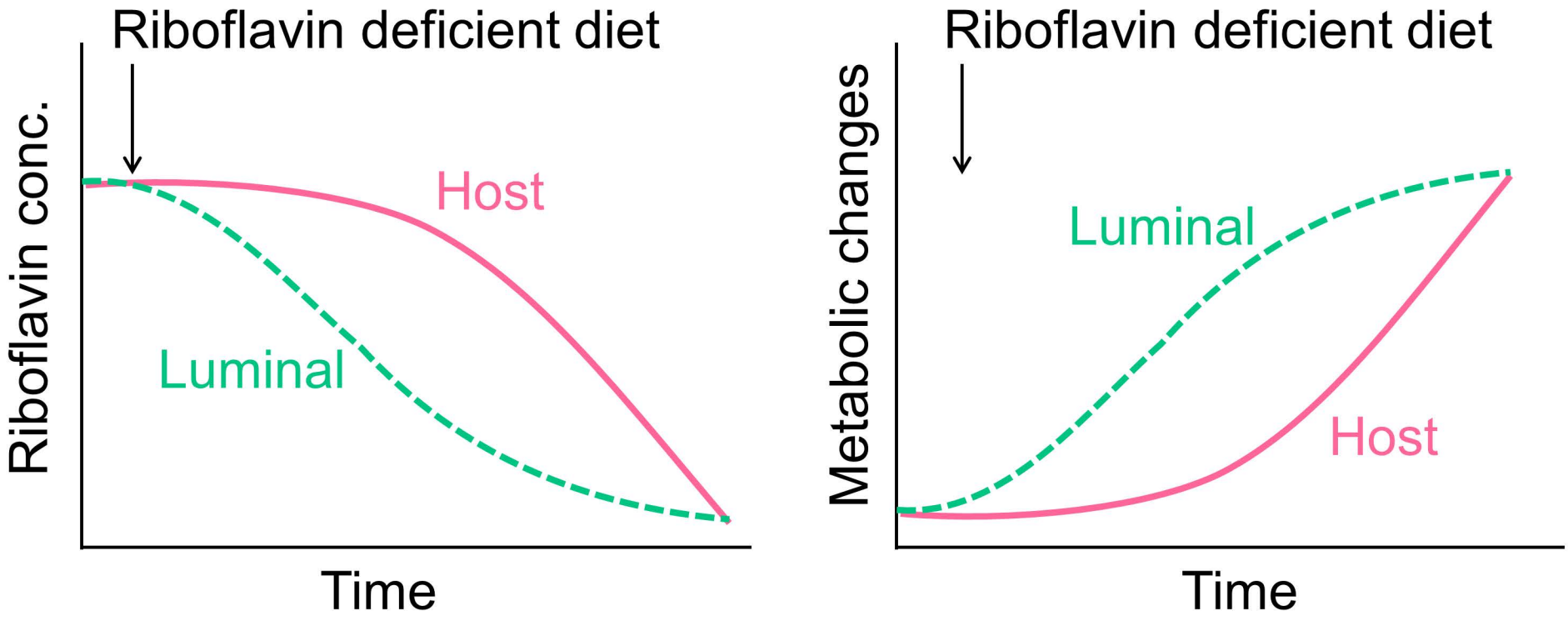
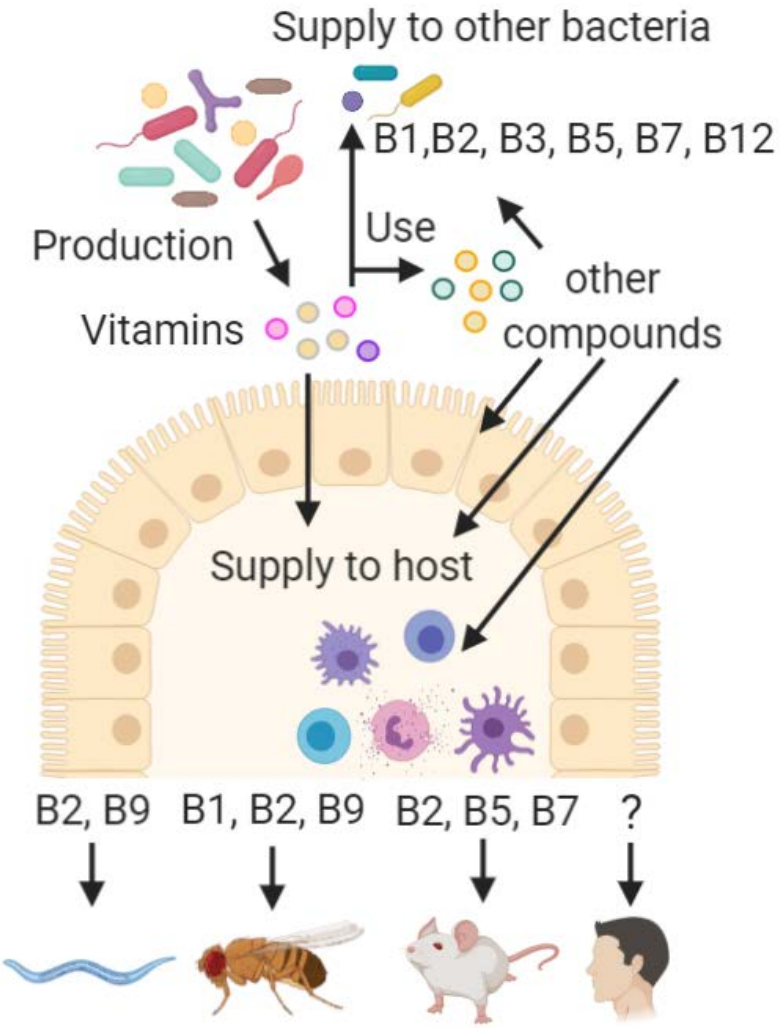

Riboflavin deficient diet
Riboflavin conc.
Host
Luminal
Time
Riboflavin deficient diet
Metabolic changes
Luminal
Host
Time

Figure 2



B-vitamins and their metabolic derivatives in the distal colon perform many important functions in the body; they act as (1) nutrients for a host and their microbiota, (2) regulators of immune cell activity and modulators of colitis, (3) mediators of drug efficacy, (4) supporters of survival, or the fitness of certain bacterium, and (5) suppressors of colonization by pathogenic bacteria.