

# 1 **Functional Roles of B-Vitamins in the Gut and Gut Microbiome**

2

3 Takashi Uebanso,\* Takaaki Shimohata, Kazuaki Mawatari, Akira Takahashi

4

5 Department of Preventive Environment and Nutrition, Institute of Biomedical Sciences, Tokushima

6 University Graduate School, Tokushima City, Japan

7

8 **Keywords:** B-vitamins/Distal gut /Gut microbiota/

9

10 **\*Corresponding author:**

11 Takashi Uebanso,

12 Department of Preventive Environment and Nutrition, Institute of Biomedical Sciences, Tokushima

13 University Graduate School, 3-18-15, Kuramoto, Tokushima 770-8503, Japan

14 E-mail: uebanso@tokushima-u.ac.jp

15 TEL: +81-88-633-9598; FAX: +81-88-633-7092.

16

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

25

26

27 **Abstract**

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

42

## 43 **1 Introduction**

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

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

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

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

## 82 **2 B-Vitamins**

83

### 84 **2.1 Vitamin B1 (thiamin)**

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

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

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

## 107 **2.2 Vitamin B2 (riboflavin)**

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

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

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

129 The pyrimidine compounds 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU) and  
130 5-(2-oxoethylideneamino)-6-D-ribitylaminouracil (5-OE-RU) are highly potent activating ligands of  
131 the mucosal-associated invariant T (MAIT)-cell (Tables 1).<sup>[49,50]</sup> Both 5-OP-RU and 5-OE-RU are  
132 generated from the riboflavin precursor 5-amino-6-D-ribitylaminouracil through the riboflavin  
133 producing pathway in various microbes. Taken together, riboflavin produced in the gut microbiota  
134 has roles in numerous hosts. These roles include functioning as an essential nutrient; or as a  
135 modulator of bacterium fitness in the gut microbiota and immune function in host. (Figure 1).

136



### 137 **2.3 Vitamin B3 (niacin)**

138 Niacin can be made from tryptophan in mammals as well as from intestinal bacteria <sup>[51–53]</sup>.

139 Mammalian colonocytes possess a carrier-mediated mechanism for the uptake of niacin <sup>[54]</sup>.

140 Exogenous expression of the human sodium-coupled monocarboxylate transporter 1 in oocytes can

141 transport both nicotinate and its structural analogs, despite substrate selectivity being low (Table

142 1).<sup>[55]</sup> Administration of microcapsules containing niacin, which release their contents at the

143 ileocolonic region, increased serum niacin concentration in a dose-dependent manner in human

144 subjects.<sup>[56]</sup>

145 Niacin acts as an agonist of cell-surface receptors, niacin receptor 1, which is also known as

146 the hydroxycarboxylic acid receptor 2 or G-protein-coupled receptor 109A. Although niacin was not

147 considered a crucial ligand of this receptor, it is expressed in colonic epithelium and its physiological

148 roles have been studied extensively.<sup>[28,57,58]</sup> A niacin deficiency results in intestinal inflammation and

149 diarrhea.<sup>[59,60]</sup> Niacin also exhibits potent antioxidant and anti-inflammatory properties and acts as a

150 modulator of intestinal barrier function and bacterial endotoxin production.<sup>[61–63]</sup> Therefore, niacin

151 has a direct impact on gut microbiota. Indeed, both tryptophan and niacin (nicotinic acid and

152 nicotinamide) treatment have been shown to revert the composition of the intestinal microbiota of

153 angiotensin I converting enzyme (peptidyl-dipeptidase A) 2 (Ace2) mutant mice (Table 1) <sup>[64]</sup>.

154 Furthermore, the intake of a microcapsule of niacin (900 to 3000 mg)—but not nicotinamide (30 to

155 300 mg)—resulted in a significant increase in the population of *Bacteroidetes* (Figure 1, Table2).<sup>[56]</sup>

156 Since *Bacteroidetes* are deficient in the enzymes nicotinamidase and nicotinamide

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

## 160 **2.4 Vitamin B5 (pantothenic acid)**

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

175

## 176 **2.5 Vitamin B6 (pyridoxine, pyridoxamine, pyridoxal)**

177 Pyridoxal 5'-phosphate (PLP), a common coenzyme form of vitamin B6, can be synthesized  
178 *de novo* via two routes.<sup>[27]</sup> The majority of *Actinobacteria*, *Bacteroidetes*, and *Proteobacteria*  
179 (approximately 50% of the 256 human gut microbiota [HGM] genomes) have at least one *de novo*  
180 biosynthetic pathway. Reduced concentrations of vitamin B6 in the colonic content of wild type  
181 (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  
182 disruptive to their gut microbiota.<sup>[73]</sup> The administration of probiotics (*Bacteroides acidifaciens*) in  
183 this context partially restored the amount of vitamin B6 in the colonic content of antibiotic-treated  
184 mice. Luminal metabolic analysis and shotgun DNA sequence analysis showed that the metabolic  
185 pathway of vitamin B6 is dynamically changed in various conditions.<sup>[74–76]</sup> The relative abundance of  
186 *Blautia*, *Coprococcus*, and *Roseburia*, which are lower in patients with schizophrenia compared with  
187 control patients, was negatively correlated with vitamin B6 metabolism-related genes (Table 2).<sup>[75]</sup>  
188 When a person resistant to atherosclerosis consumes a cholesterol-enriched diet, microbiome genes  
189 for vitamin B6 metabolism increase significantly.<sup>[74]</sup> Distal gut bacteria samples from lean  
190 individuals appear to be more involved in vitamin B6 synthesis than samples from obese people  
191 based on metaproteomics analysis (Table 2).<sup>[76]</sup> The maintenance of vitamin B6 biosynthesis in host  
192 cells are of great importance for various homeostatic processes in health and disease, including host  
193 immune responses (Figure 1).<sup>[77–79]</sup> Consistent with these reports, the estimated maximal percentage  
194 of the daily reference intake of pyridoxine is the highest (86%) of all the 8 B-vitamins<sup>[27]</sup> The carrier  
195 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.<sup>[80]</sup>

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).<sup>[81]</sup>  
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.<sup>[81]</sup>  
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).<sup>[73]</sup> 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.<sup>[82]</sup> Another study also investigated the effect of dietary vitamin B6 intake on  
210 colonic inflammation in the IL10<sup>-/-</sup> murine model of IBD.<sup>[83]</sup> 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.<sup>[84]</sup>  
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

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

232

## 233 2.6 Vitamin B7 (biotin)

234 A genomic analysis of 256 representative organisms of human gut microbiota found that  
235 40% were capable of *de novo* synthesis of the vitamin B7.<sup>[27]</sup> Studies have shown that the phylum  
236 *Bacteroidetes* (48/51 strains), *Fusobacteria* (13/14 strains), and *Proteobacteria* (29/38 strains)  
237 predicts the synthesis of biotin.<sup>[27,95]</sup> In contrast, even though *Actinobacteria* genomes lack an  
238 essential biotin biosynthesis gene, 19 of 23 (83%) of them contained a biotin transporter, indicating  
239 the need for biotin.<sup>[27]</sup> This result suggests the potential to control *Actinobacteria*-related diseases via  
240 regulating biotin availability in the gut.<sup>[96]</sup> Another study showed that four enzymes in the biotin  
241 biosynthesis pathway are overexpressed in *Bacteroides* enterotype (Table 2).<sup>[32]</sup> Absorption of biotin  
242 in both the small and large intestine occurs via a carrier-mediated process that involves the SMVT  
243 system encoded by the *SLC5A6* gene.<sup>[70,71,97–100]</sup> The colonic absorption rate of biotin measured by  
244 [14-C] biotin or the *in vivo* intestinal loop showed that post-ileal biotin absorption was 8 to 12% as  
245 efficient as the absorption of biotin after oral dosing in pig.<sup>[101,102]</sup> Lipopolysaccharides inhibit  
246 colonic biotin uptake via interference with membrane expression of its transporter.<sup>[103]</sup> On the other  
247 hand, Hayashi et al. revealed that competition for biotin utilization exists between the host and  
248 bacteria (Figure 1, Table 1).<sup>[104]</sup> *Lactobacillus murinus* consumes and reduces available biotin in the  
249 gut and antibiotic-induced dysbiosis promotes alopecia in mice fed a biotin-deficient diet (Table 1  
250 and 2).<sup>[104]</sup>

251

## 252 2.7 Vitamin B9 (folate)

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

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



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

285

## 286 **2.8 Vitamin B12 (Cobalamin)**

287           The cobalamin biosynthetic pathway is present in 42% (110/256) of the HGM genomes and  
288 can be found in all *Fusobacteria*.<sup>[27]</sup> In contrast, it is rare in *Actinobacteria* and *Proteobacteria* and  
289 half of the *Bacteroidetes* genomes are missing this biosynthetic pathway. A HGM study showed that  
290 83% of bacteria (260/313 species) encode cobalamin-dependent enzymes.<sup>[135]</sup> Intriguingly, most of  
291 these 260 species lack the genes required to synthesize cobalamin.<sup>[135]</sup> In another report, 75.9%  
292 (410/540) of the bacteria were cobalamin-utilizing organisms and only half of those (209/410)  
293 possessed the cobalamin biosynthetic pathway.<sup>[136]</sup> These genome-based analyses indicate that those  
294 bacteria rely on cobalamin-uptake mechanisms to acquire sufficient levels of it from the surrounding  
295 environment. For example, *Bacteroides thetaiotaomicron* does not encode genes involved in the  
296 cobalamin biosynthetic pathway, but rather it has three homologs of the cobalamin transporter,  
297 btuB1, btu2B, and btuB3 and a cobalamin binding factor, BtuG2 (Figure 1, Table 1).<sup>[135]</sup>

298           Recently, three cobalamin binding proteins (IF, haptocorrin, and transcobalamin) have been  
299 shown to mediate cellular cobalamin up-take in adult mammals via three receptors (i.e., IF-cbl  
300 R/cubilin, asialoglycoprotein R, and transcobalamin receptor [TC-R]). Cobalamin transport systems  
301 have been reviewed in detail elsewhere.<sup>[137–140]</sup> However, cobalamin represents only a small portion  
302 (1.4%) of the total amount in human feces.<sup>[141]</sup> Therefore, it is doubtful whether colonic-derived  
303 cobalamin has a role in nutrition of the host.

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

306 (Figure 1, Table 1) <sup>[141]</sup>. As if to have the ability to respond to it, human gut microbes likely encode at  
307 least 27 distinct corrinoid transporter families. <sup>[144,145]</sup> Supplementation of 3.94 µg/ml  
308 cyanocobalamin increased fecal cobalamin and reduced corrinoid analogs concomitant with a lower  
309 abundance of *Bacteroides* in mice (Table 2). <sup>[146]</sup> These results suggest that competition and exchanges  
310 of cobalamin and its analogs could potentially determine microbial fitness. <sup>[143,144,147]</sup> The functions of  
311 corrinoids have been studied in several reports and were thoroughly reviewed by Degnan, et  
312 al. <sup>[145,148,149]</sup> 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*. <sup>[150-153]</sup> Cobalamin uptake by  
317 the gut commensal bacteria *B. thetaiotaomicron* limits the production of Shiga-Toxin by EHEC <sup>[154]</sup>.  
318 Cobalamin also acts as an immunomodulator to promote cellular immunity. <sup>[28,155,156]</sup> These results  
319 indicated that luminal cobalamin and the availability of its analog can modulate the luminal infection.  
320

321 **3 Analytical methods**

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

329

## 330 **4 Conclusions**

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

342

343 **References**

- 344 [1] B. S. Wostmann, C. Larkin, A. Moriarty, E. Bruckner-Kardoss, *Lab Anim Sci.* **1983**, *33*, 46-50.
- 345 [2] F. Bäckhed, H. Ding, T. Wang, L. V. Hooper, Y. K. Gou, A. Nagy, C. F. Semenkovich, J. I.
- 346 Gordon, *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 15718–15723.
- 347 [3] P. J. Turnbaugh, R. E. Ley, M. A. Mahowald, V. Magrini, E. R. Mardis, J. I. Gordon, *Nature*
- 348 **2006**, *444*, 1027–1031.
- 349 [4] R. Sender, S. Fuchs, R. Milo, *Cell* **2016**, *164*, 337–340.
- 350 [5] B. O. Schroeder, F. Bäckhed, *Nat. Med.* **2016**, *22*, 1079–1089.
- 351 [6] S. V. Lynch, O. Pedersen, *N. Engl. J. Med.* **2016**, *375*, 2369–2379.
- 352 [7] R. K. Singh, H. W. Chang, D. Yan, K. M. Lee, D. Ucmak, K. Wong, M. Abrouk, B. Farahnik,
- 353 M. Nakamura, T. H. Zhu, T. Bhutani, W. Liao, *J. Transl. Med.* **2017**, *15*, 73.
- 354 [8] K. Stiemsma, L. T. Michels, *Pediatrics* **2018**, *141*, e20172437.
- 355 [9] S. I. Bechdel, Hannah, E. Honeywell, R. Adams, S. Colzege, *J. Biol. Chem.* **1928**, *80*, 231–
- 356 238.
- 357 [10] M. J. Hill, *Eur. J. Cancer Prev.* **1997**, *6*, S43-45.
- 358 [11] K. Thakur, S. K. Tomar, S. De, *Microb. Biotechnol.* **2016**, *9*, 441–451.
- 359 [12] J. G. LeBlanc, F. Chain, R. Martín, L. G. Bermúdez-Humarán, S. Courau, P. Langella, *Microb.*
- 360 *Cell Fact.* **2017**, *16*, 1–10.
- 361 [13] J. H. Martens, H. Barg, M. Warren, D. Jahn, *Appl. Microbiol. Biotechnol.* **2002**, *58*, 275–285.
- 362 [14] J. Roth, J. Lawrence, T. Bobik, *Annu. Rev. Microbiol.* **1996**, *50*, 137–181.
- 363 [15] G. P. Stozzi, M. Luca, *J. Clin. Gastroenterol.* **2008**, *42*, S179-84.
- 364 [16] H. Noda, N. Akasaka, M. Ohsugi, *J. Nutr. Sci. Vitaminol. (Tokyo).* **1994**, *40*, 181–188.
- 365 [17] A. Pompei, L. Cordisco, A. Amaretti, S. Zanoni, D. Matteuzzi, M. Rossi, *Appl. Environ.*
- 366 *Microbiol.* **2007**, *73*, 179–185.
- 367 [18] M. Kleerebezem, E. E. Vaughan, *Annu. Rev. Microbiol.* **2009**, *63*, 269–290.
- 368 [19] K. M. Shahani, R. C. Chandan, *J. Dairy Sci.* **1979**, *62*, 1685–1694.
- 369 [20] L. Alm, *J. Dairy Sci.* **1982**, *65*, 353–359.
- 370 [21] H. M. Said, *Biochem. J.* **2011**, *437*, 357–372.
- 371 [22] H. M. Said, *Am. J. Physiol. Gastrointest. Liver Physiol.* **2013**, *305*, G601-10.
- 372 [23] D. A. Rodionov, A. A. Arzamasov, M. S. Khoroshkin, S. N. Iablokov, S. A. Leyn, S. N.
- 373 Peterson, P. S. Novichkov, A. L. Osterman, *Front. Microbiol.* **2019**, *10*, 1316.
- 374 [24] S. E. Poe, G. E. J. Mitchell, D. G. Ely, *J. Anim. Sci.* **1972**, *34*, 826–829.
- 375 [25] J. G. LeBlanc, C. Milani, G. S. de Giori, F. Sesma, D. van Sinderen, M. Ventura, *Curr. Opin.*
- 376 *Biotechnol.* **2013**, *24*, 160–168.
- 377 [26] M. J. Kwak, S. K. Kwon, J. K. Yoon, J. Y. Song, J. G. Seo, M. J. Chung, J. F. Kim, *Syst. Appl.*
- 378 *Microbiol.* **2016**, *39*, 429–439.
- 379 [27] S. Magnúsdóttir, D. Ravcheev, V. De Crécy-Lagard, I. Thiele, *Front. Genet.* **2015**, *6*, 148.
- 380 [28] K. Yoshii, K. Hosomi, K. Sawane, J. Kunisawa, *Front. Nutr.* **2019**, *6*, 48.

- 381 [29] M. E. Coates, *Proc. Nutr. Soc.* **1973**, *32*, 53–58.
- 382 [30] B. S. Wostmann, *Annu. Rev. Nutr.* **1981**, *1*, 257–279.
- 383 [31] C. P. Champagne, T. A. Tompkins, N. D. Buckley, J. M. Green-Johnson, *Food Microbiol.*  
384 **2010**, *27*, 968–972.
- 385 [32] M. Arumugam, J. Raes, E. Pelletier, D. Le Paslier, T. Yamada, D. R. Mende, G. R. Fernandes,  
386 J. Tap, T. Bruls, J. M. Batto, M. Bertalan, N. Borruel, F. Casellas, L. Fernandez, L. Gautier, T.  
387 Hansen, M. Hattori, T. Hayashi, M. Kleerebezem, K. Kurokawa, M. Leclerc, F. Levenez, C.  
388 Manichanh, H. B. Nielsen, T. Nielsen, N. Pons, J. Poulain, J. Qin, T. Sicheritz-Ponten, S.  
389 Tims, D. Torrents, E. Ugarte, E. G. Zoetendal, J. Wang, F. Guarner, O. Pedersen, W. M. De  
390 Vos, S. Brunak, J. Doré, J. Weissenbach, S. D. Ehrlich, P. Bork, *Nature* **2011**, *473*, 174–180.
- 391 [33] H. M. Said, A. Ortiz, V. S. Subramanian, E. J. Neufeld, M. P. Moyer, P. K. Dudeja, *Am. J.*  
392 *Physiol. Gastrointest. Liver Physiol.* **2001**, *281*, 144–150.
- 393 [34] H. M. Said, K. Balamurugan, V. S. Subramanian, J. S. Marchant, *Am. J. Physiol. Gastrointest.*  
394 *Liver Physiol.* **2004**, *286*, 491–498.
- 395 [35] J. C. Reidling, V. S. Subramanian, P. K. Dudeja, H. M. Said, *Biochim. Biophys. Acta* **2002**,  
396 *1561*, 180–187.
- 397 [36] K. Y. Anandam, P. Srinivasan, V. S. Subramanian, H. M. Said, *Am. J. Physiol. Cell Physiol.*  
398 **2017**, *313*, C655–C663.
- 399 [37] B. Ashokkumar, J. S. Kumar, G. A. Hecht, H. M. Said, *Am. J. Physiol. Gastrointest. Liver*  
400 *Physiol.* **2009**, *297*, 825–833.
- 401 [38] J. Kunisawa, Y. Sugiura, T. Wake, T. Nagatake, H. Suzuki, R. Nagasawa, S. Shikata, K.  
402 Honda, E. Hashimoto, Y. Suzuki, M. Setou, M. Suematsu, H. Kiyono, *Cell Rep.* **2015**, *13*,  
403 122–131.
- 404 [39] D. R. Sannino, A. J. Dobson, K. Edwards, E. R. Angert, N. Buchon, *MBio* **2018**, *9*, e00155-18.
- 405 [40] Z. A. Costliow, P. H. Degnan, *mSystems* **2017**, *2*, e00116-17.
- 406 [41] V. S. Subramanian, S. B. Subramanya, A. Ghosal, H. M. Said, *Am. J. Physiol. Cell Physiol.*  
407 **2013**, *305*, C539-546.
- 408 [42] V. S. Subramanian, N. Lambrecht, C. Lytle, H. M. Said, *Am. J. Physiol. - Gastrointest. Liver*  
409 *Physiol.* **2016**, *310*, G285–G293.
- 410 [43] T. Uebanso, A. Yoshimoto, S. Aizawa, M. Nakamura, R. Masuda, T. Shimohata, K. Mawatari,  
411 A. Takahashi, *Nutrients* **2020**, *12*, 736.
- 412 [44] C.-N. W. Adam, D. Adam, A. Douglas, *J. Exp. Biol.* **2014**, *217*, 1894–1901.
- 413 [45] B. Qi, M. Kniazeva, M. Han, *Elife* **2017**, *6*, e26243.
- 414 [46] M. T. Khan, S. H. Duncan, A. J. M. Stams, J. M. Van Dijn, H. J. Flint, H. J. M. Harmsen,  
415 *ISME J.* **2012**, *6*, 1578–1585.
- 416 [47] R. E. Steinert, M. S. Sadabad, H. J. M. Harmsen, P. Weber, *Eur. J. Clin. Nutr.* **2016**, *70*, 1348–  
417 53.
- 418 [48] A. Heinken, M. T. Khan, G. Paglia, D. A. Rodionov, H. J. M. Harmsen, I. Thiele, *J. Bacteriol.*  
419 **2014**, *196*, 3289–3302.

- 420 [49] A. J. Corbett, S. B. G. Eckle, R. W. Birkinshaw, L. Liu, O. Patel, J. Mahony, Z. Chen, R.  
421 Reantragoon, B. Meehan, H. Cao, N. A. Williamson, R. A. Strugnell, D. Van Sinderen, J. Y.  
422 W. Mak, D. P. Fairlie, L. Kjer-Nielsen, J. Rossjohn, J. McCluskey, *Nature* **2014**, *509*, 361–  
423 365.
- 424 [50] H. E. G. McWilliam, J. A. Villadangos, *Trends Immunol.* **2017**, *38*, 679–689.
- 425 [51] O. Kurnasov, V. Goral, K. Colabroy, S. Gerdes, S. Anantha, S. Osterman, T. P. Begley, *Chem.*  
426 *Biol.* **2003**, *10*, 1195–204.
- 427 [52] M. E. Coates, J. E. Ford, G. F. Harrison, *Br. J. Nutr.* **1968**, *22*, 493–500.
- 428 [53] F. Gazzaniga, R. Stebbins, S. Z. Chang, M. A. McPeck, C. Brenner, *Microbiol. Mol. Biol. Rev.*  
429 **2009**, *73*, 529–541.
- 430 [54] J. S. Kumar, V. S. Subramanian, R. Kapadia, M. L. Kashyap, H. M. Said, *Am. J. Physiol.*  
431 *Gastrointest. Liver Physiol.* **2013**, *305*, G207-13.
- 432 [55] E. Gopal, S. Miyauchi, P. M. Martin, S. Ananth, P. Roon, S. B. Smith, V. Ganapathy, *Pharm.*  
433 *Res.* **2007**, *24*, 575–584.
- 434 [56] D. Fangmann, E. M. Theismann, K. Turk, D. M. Schulte, I. Relling, K. Hartmann, J. K.  
435 Keppler, J. R. Knipp, A. Rehman, F. A. Heinsen, A. Franke, L. Lenk, S. Freitag-Wolf, E.  
436 Appel, S. Gorb, C. Brenner, D. Seegert, G. H. Waetzig, P. Rosenstiel, S. Schreiber, K.  
437 Schwarz, M. Laudes, *Diabetes Care* **2018**, *41*, 398–405.
- 438 [57] V. Ganapathy, M. Thangaraju, P. D. Prasad, P. M. Martin, N. Singh, *Curr. Opin. Pharmacol.*  
439 **2013**, *13*, 869–874.
- 440 [58] S. Offermanns, *Trends Endocrinol. Metab.* **2017**, *28*, 227–236.
- 441 [59] J. Hegyi, R. A. Schwartz, V. Hegyi, *Int. J. Dermatol.* **2004**, *43*, 1–5.
- 442 [60] I. Segal, L. O. Tim, A. Demetriou, A. Paterson, M. Hale, M. Leros, *Int. J. Colorectal Dis.*  
443 **1986**, *1*, 238–243.
- 444 [61] F. Karpe, K. N. Frayn, *Lancet* **2004**, *363*, 1892–1894.
- 445 [62] V. S. Kamanna, M. L. Kashyap, *Am. J. Cardiol.* **2008**, *101*, 20B-26B.
- 446 [63] W. Zhong, Q. Li, W. Zhang, Q. Sun, X. Sun, Z. Zhou, *Biomolecules* **2015**, *5*, 2643–2658.
- 447 [64] T. Hashimoto, T. Perlot, A. Rehman, J. Trichereau, H. Ishiguro, M. Paolino, V. Sigl, T.  
448 Hanada, R. Hanada, S. Lipinski, B. Wild, S. M. R. Camargo, D. Singer, A. Richter, K. Kuba,  
449 A. Fukamizu, S. Schreiber, H. Clevers, F. Verrey, P. Rosenstiel, J. M. Penninger, *Nature* **2012**,  
450 *487*, 477–481.
- 451 [65] J. M. Carrothers, M. A. York, S. L. Brooker, K. A. Lackey, J. E. Williams, B. Shafii, W. J.  
452 Price, M. L. Settles, M. A. McGuire, M. K. McGuire, *J. Nutr.* **2015**, *145*, 2379–2388.
- 453 [66] C. Yao, J. Chou, T. Wang, H. Zhao, B. Zhang, *Front. Microbiol.* **2018**, *9*, 1194.
- 454 [67] H. Khan, S. H. Flint, P. L. Yu, *J. Appl. Microbiol.* **2013**, *114*, 1092–1102.
- 455 [68] V. Ragaller, P. Lebzien, K. H. Südekum, L. Hüther, G. Flachowsky, *J. Anim. Physiol. Anim.*  
456 *Nutr. (Berl).* **2011**, *95*, 6–16.
- 457 [69] X. Wang, J. Wang, B. Rao, L. I. Deng, *Exp. Ther. Med.* **2017**, *13*, 2848–2854.
- 458 [70] P. D. Prasad, H. Wang, W. Huang, Y. J. Fei, F. H. Leibach, L. D. Devoe, V. Ganapathy, *Arch.*



- 459 *Biochem. Biophys.* **1999**, *366*, 95–106.
- 460 [71] A. Ghosal, N. Lambrecht, S. B. Subramanya, R. Kapadia, H. M. Said, *Am. J. Physiol.*  
461 *Gastrointest. Liver Physiol.* **2013**, *304*, 64–71.
- 462 [72] E. D. Stein, J. M. Diamond, *J. Nutr.* **1989**, *119*, 1973–83.
- 463 [73] T. Miki, R. Goto, M. Fujimoto, N. Okada, W. D. Hardt, *Cell Host Microbe* **2017**, *21*, 195–207.
- 464 [74] S. Liu, H. M. Tun, F. C. Leung, D. C. Bennett, H. Zhang, K. M. Cheng, *Sci. Rep.* **2018**, *8*,  
465 2381.
- 466 [75] Y. Shen, J. Xu, Z. Li, Y. Huang, Y. Yuan, J. Wang, M. Zhang, S. Hu, Y. Liang, *Schizophr.*  
467 *Res.* **2018**, *197*, 470–477.
- 468 [76] M. Ferrer, A. Ruiz, F. Lanza, S. B. Haange, A. Oberbach, H. Till, R. Bargiela, C. Campoy, M.  
469 T. Segura, M. Richter, M. von Bergen, J. Seifert, A. Suarez, *Environ. Microbiol.* **2013**, *15*,  
470 211–226.
- 471 [77] L. S. Harbige, *Nutr. Health* **1996**, *10*, 285–312.
- 472 [78] S. N. Meydani, J. D. Ribaya-Mercado, R. M. Russell, N. Sahyoun, F. D. Morrow, S. N.  
473 Gershoff, *Am. J. Clin. Nutr.* **1991**, *53*, 1275–80.
- 474 [79] L. C. Rall, S. N. Meydani, *Nutr. Rev.* **1993**, *51*, 217–25.
- 475 [80] Z. M. Said, V. S. Subramanian, N. D. Vaziri, H. M. Said, *Am. J. Physiol. Cell Physiol.* **2008**,  
476 *294*, 1192–1197.
- 477 [81] T. A. Scott, L. M. Quintaneiro, P. Norvaisas, P. P. Lui, M. P. Wilson, K. Y. Leung, L. Herrera-  
478 Dominguez, S. Sudiwala, A. Pessia, P. T. Clayton, K. Bryson, V. Velagapudi, P. B. Mills, A.  
479 Typas, N. D. E. Greene, F. Cabreiro, *Cell* **2017**, *169*, 442–456.
- 480 [82] N. M. Benight, B. Stoll, S. Chacko, V. R. da Silva, J. C. Marini, J. F. Gregory, S. P. Stabler, D.  
481 G. Burrin, *Am. J. Physiol. Gastrointest. Liver Physiol.* **2011**, *301*, G249-259.
- 482 [83] J. Selhub, A. Byun, Z. Liu, J. B. Mason, R. T. Bronson, J. W. Crott, *J. Nutr. Biochem.* **2013**,  
483 *24*, 2138–2143.
- 484 [84] K. Vagianos, S. Bector, J. McConnell, C. N. Bernstein, *J. Parenter. Enter. Nutr.* **2007**, *31*,  
485 311–9.
- 486 [85] M. N. Kazarinoff, D. B. McCormick, *J. Biol. Chem.* **1975**, *250*, 3436–42.
- 487 [86] T. D. Hubbard, I. A. Murray, G. H. Perdew, *Drug Metab. Dispos.* **2015**, *43*, 1522–1535.
- 488 [87] S. S. Un-Ho Jin, Syng-Ook Lee, Gautham Sridharan, Kyongbum Lee, Laurie A Davidson,  
489 Arul Jayaraman, Robert S Chapkin, Robert Alaniz, *Mol. Pharmacol.* **2014**, *85*, 777–88.
- 490 [88] B. Lamas, M. L. Richard, V. Leducq, H. P. Pham, M. L. Michel, G. Da Costa, C. Bridonneau,  
491 S. Jegou, T. W. Hoffmann, J. M. Natividad, L. Brot, S. Taleb, A. Couturier-Maillard, I. Nion-  
492 Larmurier, F. Merabtene, P. Seksik, A. Bourrier, J. Cosnes, B. Ryffel, L. Beaugerie, J. M.  
493 Launay, P. Langella, R. J. Xavier, H. Sokol, *Nat. Med.* **2016**, *22*, 598–605.
- 494 [89] R. Aoki, A. Aoki-Yoshida, C. Suzuki, Y. Takayama, *J. Immunol.* **2018**, *201*, 3683–3693.
- 495 [90] M. Wlodarska, C. Luo, R. Kolde, E. D’Hennezel, J. W. Annand, C. E. Heim, P. Krastel, E. K.  
496 Schmitt, A. S. Omar, E. A. Creasey, A. L. Garner, S. Mohammadi, D. J. O’Connell, S.  
497 Abubucker, T. D. Arthur, E. A. Franzosa, C. Huttenhower, L. O. Murphy, H. J. Haiser, H.

- 498 Vlamakis, J. A. Porter, R. J. Xavier, *Cell Host Microbe* **2017**, *22*, 25–37.
- 499 [91] C. A. Opitz, U. M. Litzenburger, F. Sahm, M. Ott, I. Tritschler, S. Trump, T. Schumacher, L.  
500 Jestaedt, D. Schrenk, M. Weller, M. Jugold, G. J. Guillemin, C. L. Miller, C. Lutz, B.  
501 Radlwimmer, I. Lehmann, A. Von Deimling, W. Wick, M. Platten, *Nature* **2011**, *478*, 197–  
502 203.
- 503 [92] T. Takamura, D. Harama, S. Matsuoka, N. Shimokawa, Y. Nakamura, K. Okumura, H.  
504 Ogawa, M. Kitamura, A. Nakao, *Immunol. Cell Biol.* **2010**, *88*, 685–689.
- 505 [93] K. Furumatsu, S. Nishiumi, Y. Kawano, M. Ooi, T. Yoshie, Y. Shiomi, H. Kutsumi, H.  
506 Ashida, Y. Fujii-Kuriyama, T. Azuma, M. Yoshida, *Dig. Dis. Sci.* **2011**, *56*, 2532–2544.
- 507 [94] J. Islam, S. Sato, K. Watanabe, T. Watanabe, Ardiansyah, K. Hirahara, Y. Aoyama, S. Tomita,  
508 H. Aso, M. Komai, H. Shirakawa, *J. Nutr. Biochem.* **2017**, *42*, 43–50.
- 509 [95] E. G. Zoetendal, J. Raes, B. Van Den Bogert, M. Arumugam, C. C. Booiijink, F. J. Troost, P.  
510 Bork, M. Wels, W. M. De Vos, M. Kleerebezem, *ISME J.* **2012**, *6*, 1415–1426.
- 511 [96] C. Binda, L. R. Lopetuso, G. Rizzatti, G. Gibiino, V. Cennamo, A. Gasbarrini, *Dig. Liver Dis.*  
512 **2018**, *50*, 421–428.
- 513 [97] H. M. Said, A. Ortiz, E. McCloud, D. Dyer, M. P. Moyer, S. Rubin, *Am. J. Physiol. Cell*  
514 *Physiol.* **1998**, *275*, C1365–1371.
- 515 [98] K. Balamurugan, A. Ortiz, H. M. Said, *Am. J. Physiol. - Gastrointest. Liver Physiol.* **2003**,  
516 *285*, 73–77.
- 517 [99] M. Hamid, *J. Nutr.* **2009**, *1*, 158–162.
- 518 [100] S. Sabui, J. Skupsky, R. Kapadia, K. Cogburn, N. W. Lambrecht, A. Agrawal, H. M. Said, *Am.*  
519 *J. Physiol. Gastrointest. Liver Physiol.* **2019**, *317*, G518–G530.
- 520 [101] J. S. Kopinski, J. Leibholz, J. Leibholz, R. J. Love, *Br. J. Nutr.* **1989**, *62*, 781–789.
- 521 [102] B. B. Bowman, I. H. Rosenberg, *J. Nutr.* **1987**, *117*, 2121–6.
- 522 [103] R. Lakhan, H. M. Said, *Am. J. Physiol. Cell Physiol.* **2017**, *312*, C376–C384.
- 523 [104] A. Hayashi, Y. Mikami, K. Miyamoto, N. Kamada, T. Sato, S. Mizuno, M. Naganuma, T.  
524 Teratani, R. Aoki, S. Fukuda, W. Suda, M. Hattori, M. Amagai, M. Ohyama, T. Kanai, *Cell*  
525 *Rep.* **2017**, *20*, 1513–1524.
- 526 [105] A. Pompei, L. Cordisco, A. Amaretti, S. Zanoni, S. Raimondi, D. Matteuzzi, M. Rossi, *J. Nutr.*  
527 **2007**, *137*, 2742–2746.
- 528 [106] S. Aufreiter, J. H. Kim, D. L. O'Connor, *J. Nutr.* **2011**, *141*, 366–372.
- 529 [107] J. G. Leblanc, J. E. Laiño, M. J. del Valle, V. Vannini, D. van Sinderen, M. P. Taranto, G. F.  
530 de Valdez, G. S. de Giori, F. Sesma, *J. Appl. Microbiol.* **2011**, *111*, 1297–1309.
- 531 [108] M. Rossi, A. Amaretti, S. Raimondi, *Nutrients* **2011**, *3*, 118–134.
- 532 [109] H. Sugahara, T. Odamaki, N. Hashikura, F. Abe, J. Xiao, *Biosci. Microbiota, Food Heal.*  
533 **2015**, *34*, 87–93.
- 534 [110] N. Rong, J. Selhub, B. R. Goldin, I. H. Rosenberg, *J. Nutr.* **1991**, *121*, 1955–9.
- 535 [111] F. M. Asrar, D. L. O'Connor, *J. Nutr. Biochem.* **2005**, *16*, 587–593.
- 536 [112] S. Aufreiter, J. F. Gregory, C. M. Pfeiffer, Z. Fazili, Y. I. Kim, N. Marcon, P. Kamalapor, P.

- 537 B. Pencharz, D. L. O'Connor, *Am. J. Clin. Nutr.* **2009**, *90*, 116–123.
- 538 [113] A. Lakoff, Z. Fazili, S. Aufreiter, C. M. Pfeiffer, B. Connolly, J. F. Gregory, P. B. Pencharz,  
539 D. L. O'Connor, *Am. J. Clin. Nutr.* **2014**, *100*, 1278–1286.
- 540 [114] P. K. Dudeja, S. A. Torania, H. M. Said, *Am. J. Physiol. Gastrointest. Liver Physiol.* **1997**,  
541 *272*, G1408-15.
- 542 [115] P. K. Dudeja, A. Kode, M. Alnounou, S. Tyagi, S. Torania, V. S. Subramanian, H. M. Said,  
543 *Am. J. Physiol. Gastrointest. Liver Physiol.* **2001**, *281*, G54-60.
- 544 [116] B. L. Urquhart, J. C. Gregor, N. Chande, M. J. Knauer, R. G. Tirona, R. B. Kim, *Am. J.*  
545 *Physiol. Gastrointest. Liver Physiol.* **2010**, *298*, G248-254.
- 546 [117] M. Hinken, S. Halwachs, C. Kneuer, W. Honscha, *Eur. J. Histochem.* **2011**, *55*, 11–18.
- 547 [118] Y. Wang, R. Zhao, R. G. Russell, I. D. Goldman, *Biochim. Biophys. Acta* **2001**, *1513*, 49–54.
- 548 [119] A. Qiu, M. Jansen, A. Sakaris, S. H. Min, S. Chattopadhyay, E. Tsai, C. Sandoval, R. Zhao, M.  
549 H. Akabas, I. D. Goldman, *Cell* **2006**, *127*, 917–928.
- 550 [120] L. H. Matherly, M. R. Wilson, Z. Hou, *Drug Metab. Dispos.* **2014**, *42*, 632–649.
- 551 [121] Z. Hou, L. H. Matherly, *Curr. Top. Membr.* **2014**, *73*, 175–204.
- 552 [122] B. Ashokkumar, Z. M. Mohammed, N. D. Vaziri, H. M. Said, *Am. J. Clin. Nutr.* **2007**, *86*,  
553 159–166.
- 554 [123] A. Mardinoglu, H. Wu, E. Bjornson, C. Zhang, A. Hakkarainen, S. M. Räsänen, S. Lee, R. M.  
555 Mancina, M. Bergentall, K. H. Pietiläinen, S. Söderlund, N. Matikainen, M. Ståhlman, P. O.  
556 Bergh, M. Adiels, B. D. Piening, M. Granér, N. Lundbom, K. J. Williams, S. Romeo, J.  
557 Nielsen, M. Snyder, M. Uhlén, G. Bergström, R. Perkins, H. U. Marschall, F. Bäckhed, M. R.  
558 Taskinen, J. Borén, *Cell Metab.* **2018**, *27*, 559-571.e5.
- 559 [124] B. Virk, J. Jia, C. A. Maynard, A. Raimundo, J. Lefebvre, S. A. Richards, N. Chetina, Y.  
560 Liang, N. Helliwell, M. Cipinska, D. Weinkove, *Cell Rep.* **2016**, *14*, 1611–1620.
- 561 [125] F. Cabreiro, C. Au, K. Y. Leung, N. Vergara-Irigaray, H. M. Cochemé, T. Noori, D.  
562 Weinkove, E. Schuster, N. D. E. Greene, D. Gems, *Cell* **2013**, *153*, 228–239.
- 563 [126] R. I. Clark, A. Salazar, R. Yamada, S. Fitz-Gibbon, M. Morselli, J. Alcaraz, A. Rana, M. Rera,  
564 M. Pellegrini, W. W. Ja, D. W. Walker, *Cell Rep.* **2015**, *12*, 1656–1667.
- 565 [127] C. M. Thomas, D. M. A. Saulnier, J. K. Spinler, P. Hemarajata, C. Gao, S. E. Jones, A.  
566 Grimm, M. A. Balderas, M. D. Burstein, C. Morra, D. Roeth, M. Kalkum, J. Versalovic,  
567 *Microbiologyopen* **2016**, *5*, 802–818.
- 568 [128] M. A. Engevik, C. N. Morra, D. Röth, K. Engevik, J. K. Spinler, S. Devaraj, S. E. Crawford,  
569 M. K. Estes, M. Kalkum, J. Versalovic, *Front. Microbiol.* **2019**, *10*, 2305.
- 570 [129] H. H. J. Backus, H. M. Pinedo, D. Wouters, J. M. Padrón, N. Molders, C. L. Van Der Wilt, C.  
571 J. Van Groeningen, G. Jansen, G. J. Peters, *Int. J. Cancer* **2000**, *87*, 771–778.
- 572 [130] Y. I. Kim, *J Nutr* **2003**, *133*, 3731S-3739S.
- 573 [131] E. Odin, Y. Wettergren, S. Nilsson, R. Willén, G. Carlsson, C. P. Spears, L. Larsson, B.  
574 Gustavsson, *Clin. Cancer Res.* **2003**, *9*, 6012–6019.
- 575 [132] S. Handali, E. Moghimipour, M. Kouchak, Z. Ramezani, M. Amini, K. A. Angali, S. Saremy,

- 576 F. A. Dorkoosh, M. Rezaei, *Life Sci.* **2019**, *227*, 39–50.
- 577 [133] M. L. Cravo, J. B. Mason, J. Selhub, I. H. Rosenberg, *Am. J. Clin. Nutr.* **1991**, *53*, 1450–54.
- 578 [134] L. Kjer-Nielsen, O. Patel, A. J. Corbett, J. Le Nours, B. Meehan, L. Liu, M. Bhati, Z. Chen, L.  
579 Kostenko, R. Reantragoon, N. A. Williamson, A. W. Purcell, N. L. Dudek, M. J. McConville,  
580 R. A. J. O’Hair, G. N. Khairallah, D. I. Godfrey, D. P. Fairlie, J. Rossjohn, J. McCluskey,  
581 *Nature* **2012**, *491*, 717–723.
- 582 [135] P. H. Degan, N. A. Barry, K. C. Mok, M. E. Taga, A. L. Goodman, *Cell Host Microbe* **2014**,  
583 *15*, 47–57.
- 584 [136] Y. Zhang, D. A. Rodionov, M. S. Gelfand, V. N. Gladyshev, *BMC Genomics* **2009**, *10*, 78.
- 585 [137] G. J. Russell-Jones, D. H. Alpers, *Pharm. Biotechnol.* **1999**, *12*, 493–520.
- 586 [138] R. Kozyraki, M. Kristiansen, A. Silaharoglu, C. Hansen, C. Jacobsen, N. Tommerup, P. J.  
587 Verroust, S. K. Moestrup, *Blood* **1998**, *91*, 3593–600.
- 588 [139] S. A. Frank, *Ecol. Evol.* **2017**, *7*, 10175–10195.
- 589 [140] C. B. Juul, S. N. Fedosov, E. Nexo, C. W. Heegaard, *Mol. Biol. Cell* **2019**, *30*, 467–477.
- 590 [141] R. H. Allen, S. P. Stabler, *Am. J. Clin. Nutr.* **2008**, *87*, 1324–1335.
- 591 [142] D. H. Guimarães, A. Weber, I. Klaiber, B. Vogler, P. Renz, *Arch. Microbiol.* **1994**, *162*, 272–  
592 276.
- 593 [143] B. Kräutler, W. Fieber, S. Ostermann, M. Fasching, K. H. Ongania, K. Gruber, C. Kratky, C.  
594 Mikl, A. Siebert, G. Diekert, *Helv. Chim. Acta* **2003**, *86*, 3698–3716.
- 595 [144] Y. Men, E. C. Seth, S. Yi, T. S. Crofts, R. H. Allen, M. E. Taga, L. Alvarez-Cohen, *Environ.*  
596 *Microbiol.* **2015**, *17*, 4873–4884.
- 597 [145] P. H. Degan, M. E. Taga, A. L. Goodman, *Cell Metab.* **2014**, *20*, 769–778.
- 598 [146] C. J. Kelly, E. E. Alexeev, L. Farb, T. W. Vickery, L. Zheng, C. Eric L, D. A. Kitzenberg, K.  
599 D. Battista, D. J. Kominsky, C. E. Robertson, D. N. Frank, S. P. Stabler, S. P. Colgan, *Gut*  
600 *Microbes* **2019**, *10*, 654–662.
- 601 [147] A. L. Goodman, N. P. McNulty, Y. Zhao, D. Leip, R. D. Mitra, C. A. Lozupone, R. Knight, J.  
602 I. Gordon, *Cell Host Microbe* **2009**, *6*, 279–289.
- 603 [148] S. Keller, M. Ruetz, C. Kunze, B. Kräutler, G. Diekert, T. Schubert, *Environ. Microbiol.* **2014**,  
604 *16*, 3361–3369.
- 605 [149] K. C. Mok, M. E. Taga, *J. Bacteriol.* **2013**, *195*, 1902–1911.
- 606 [150] C. J. Anderson, D. E. Clark, M. Adli, M. M. Kendall, *PLoS Pathog.* **2015**, *11*, e1005278.
- 607 [151] P. Thiennimitr, S. E. Winter, M. G. Winter, M. N. Xavier, V. Tolstikov, D. L. Huseby, T.  
608 Sterzenbach, R. M. Tsohis, J. R. Roth, A. J. Bäuml, *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*,  
609 17480–17485.
- 610 [152] M. M. Kendall, C. C. Gruber, C. T. Parker, V. Sperandio, *MBio* **2012**, *3*, e00050-12.
- 611 [153] D. H. Luzader, D. E. Clark, L. A. Gonyar, M. M. Kendall, *J. Bacteriol.* **2013**, *195*, 4947–4953.
- 612 [154] C. Cordonnier, G. Le Bihan, J. G. Emond-Rheault, A. Garrivier, J. Harel, G. Jubelin, *Toxins*  
613 *(Basel)*. **2016**, *8*, 14.
- 614 [155] J. Tamura, K. Kubota, H. Murakami, M. Sawamura, T. Matsushima, T. Tamura, T. Saitoh, H.

- 615 Kurabayshi, T. Naruse, *Clin. Exp. Immunol.* **1999**, *116*, 28–32.
- 616 [156] H. Shapiro, C. A. Thaiss, M. Levy, E. Elinav, *Curr. Opin. Immunol.* **2014**, *30*, 54–62.
- 617
- 618
- 619

620 **Author contributions**

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

624

625 **Acknowledgements**

626 This work was financially supported by JSPS KAKENHI Grant Number 20K11624. The authors  
627 gratefully acknowledge the excellent assistance provided by Yumi Harada. T.U. is grateful to family,  
628 laboratory members, and the Jokyo-kai for their support.

629

630 **Conflicts of Interest:** The authors declare no conflicts of interest.

631

632 **Figure Legends**

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

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

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

642 Table 1 Importance of B-vitamin in distal gut

Vitamin	DRI <sup>a</sup>	Colon transporter (official symbol) <sup>b</sup>	Importance of B vitamin in distal gut
B1, thiamin	2.3%	THTR1 (SLC19A2) THTR2 (SLC19A3)	<ul style="list-style-type: none"> <li>• Supplies <i>D. melanogaster</i> with nutrients<sup>*[39]</sup></li> </ul>
B2, riboflavin	2.8%	RFVT3 (SLC52A3)	<ul style="list-style-type: none"> <li>• Supplies mice with nutrients<sup>[43]</sup></li> <li>• Supports survival of <i>D. melanogaster</i><sup>*</sup> and <i>C. elegans</i><sup>*[44,45]</sup></li> <li>• Supports electron transfer in oxygen sensitive bacteria<sup>[46,47]</sup></li> <li>• Metabolites activate MAIT-cells by derived from a riboflavin precursor<sup>[49,50]</sup></li> </ul>
B3, niacin	27%	SMCT1 (SLC5A8), GPR109A (HCAR2)	<ul style="list-style-type: none"> <li>• Helps growth of Bacteroidetes<sup>[53]</sup></li> </ul>
B5, pantothenic acid	0.078%	SMVT (SLC5A6)	<ul style="list-style-type: none"> <li>• Nutrient supply for microbes<sup>[66–68]</sup> and mice<sup>[72]</sup></li> </ul>
B6, pyridoxine, pyridoxamine, pyridoxal	86%	N.D.	<ul style="list-style-type: none"> <li>• Manipulates the therapeutic potential of 5-FU in <i>C. elegans</i><sup>*[81]</sup></li> <li>• Suppresses colonization of pathogenic bacteria in mice<sup>[73]</sup></li> <li>• Modulates colitis experimentally in mice<sup>[74–86]</sup></li> </ul>
B7, biotin	4.5%	SMVT, (SLC5A6)	<ul style="list-style-type: none"> <li>• Supplies mice with nutrients<sup>[104]</sup></li> </ul>
B9, folate	37%	hPCFT (SLC46A1) Rfc1 (SLC19A1)	<ul style="list-style-type: none"> <li>• Folate metabolism in the gut microbiota modulates host aging and life span<sup>[124,125]</sup></li> <li>• Helps the anti-inflammatory function of <i>Lactobacillus reuteri</i><sup>[127]</sup></li> <li>• Folate and folate-derived metabolites regulate immune function<sup>[28,135]</sup></li> </ul>
B12, cobalamin	31%	N.D.	<ul style="list-style-type: none"> <li>• Provides other functional corrinoids<sup>[136,145, 147-149]</sup></li> <li>• Cobalamin and cobalamin derived metabolites regulate microbial fitness and pathogenicity<sup>[136,143,147]</sup></li> </ul>

643 a) DRI were estimated in Magnúsdóttir S, et al.<sup>[27]</sup> 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.



645 b) Mammalian vitamin transporter in the colon was described. N. D.; not determined.  
646 \* Depends on the differences in organ structure between insects and mammals, the results from *D. melanogaster* and *C. elegans* did not apply  
647 directly to mammals.  
648 5-FU, 5-fluorouracil; Ace2, angiotensin I converting enzyme (peptidyl-dipeptidase A) 2; DRI, dietary reference intake; HCAR2, hydroxycarboxylic  
649 acid receptor 2; hPCFT, human proton-coupled folate transporter; MAIT-cell, mucosal-associated invariant T-Cell; Rfc1, reduced folate carrier;  
650 RFVT3, riboflavin transporter; SMCT1, sodium-coupled monocarboxylate transporter 1; SMVT, sodium-dependent multivitamin transporter;  
651 THTR1, thiamin transporter1; THTR2, thiamin transporter 2.

Vitamin	Relationship between B-vitamins and gut bacteria
B1, thiamin	<ul style="list-style-type: none"> <li>• Biosynthesis pathway of thiamin were overrepresented in <i>Prevotella</i> enterotype<sup>[32]</sup>.</li> <li>• Critical for the growth of <i>B. thetaiotaomicron</i> <i>in vitro</i><sup>[40]</sup></li> </ul>
B2, riboflavin	<ul style="list-style-type: none"> <li>• Dietary riboflavin supplementation increased <i>F. prausnitzii</i><sup>[47]</sup></li> <li>• Open questions</li> </ul>
B3, niacin	<ul style="list-style-type: none"> <li>• A microcapsule of niacin (900 to 3000 mg) resulted in a significant increase in the population of <i>Bacteroidetes</i><sup>[56]</sup></li> <li>• Nicotinamide supplementation with drink improved the composition of the gut microbiota in Ace2<sup>(-/-)</sup> mice<sup>[64]</sup></li> </ul>
B5, pantothenic acid	<ul style="list-style-type: none"> <li>• Increased intake of pantothenic acid was related to an increased relative abundance of <i>Actinobacteria</i><sup>[65]</sup></li> <li>• <i>Lactobacillus</i> spp., <i>Streptococcus</i> spp., and <i>Enterococcus</i> spp., required pantothenic acid for their growth <i>in vitro</i> <sup>[66-68]</sup>.</li> <li>• Open questions</li> </ul>
B6, pyridoxine, pyridoxamine, pyridoxal	<ul style="list-style-type: none"> <li>• The relative abundance of <i>Blautia</i>, <i>Coprococcus</i>, and <i>Roseburia</i> was negatively correlated with vitamin B6 metabolism-related genes<sup>[75]</sup>.</li> <li>• Distal gut bacteria samples from lean individuals appear to be more involved in vitamin B6 synthesis concomitant with lower ratio of <i>Firmicutes/Bacteroides</i> <sup>[76]</sup>.</li> </ul>
B7, biotin	<ul style="list-style-type: none"> <li>• Biotin biosynthesis pathway are overexpressed in <i>Bacteroides</i> enterotype<sup>[32]</sup>.</li> <li>• Critical for the growth of <i>Lactobacillus murinus</i> <sup>[104]</sup></li> </ul>
B9, folate	<ul style="list-style-type: none"> <li>• Abundance of <i>Bifidobacterium</i> and <i>Lactobacillus</i> was positively associated with folate status<sup>[105]</sup>.</li> <li>• Increased total aerobic bacteria was related to an increased total amount of folate in the intestinal content<sup>[106]</sup>.</li> </ul>
B12, cobalamin	<ul style="list-style-type: none"> <li>• Supplies nutrients for <i>B. thetaiotaomicron</i> <i>in vitro</i> <sup>[135]</sup></li> <li>• Supplementation of cyanocobalamin increased fecal cobalamin concomitant with a lower abundance of <i>Bacteroides</i><sup>[146]</sup>.</li> </ul>

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

---

1. Mass spectrometry (MS):	656
– MS can distinguish B-vitamins from metabolic derivatives (e.g., cobalamin from other corrinoids).	657
– 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.	

---

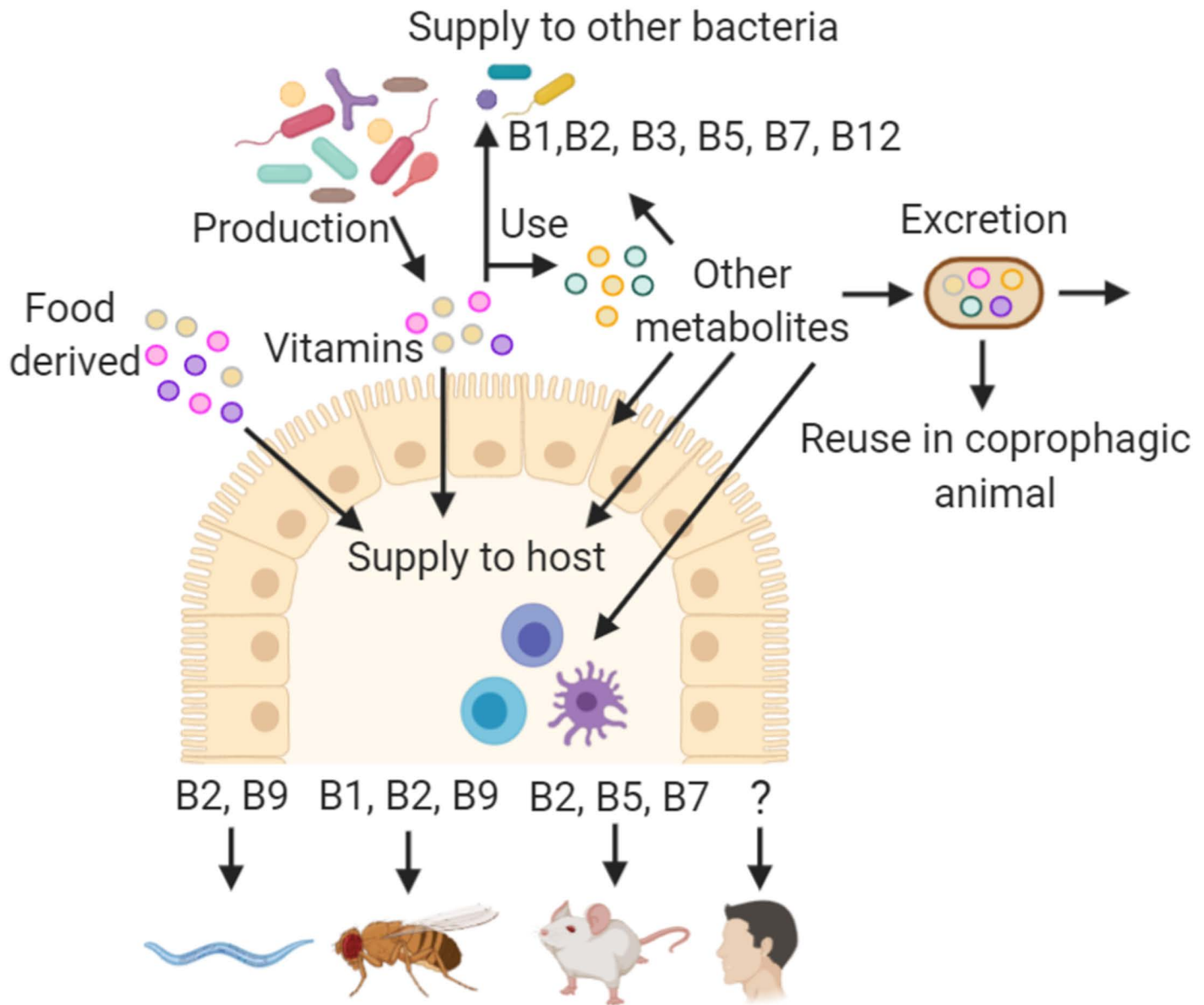


Figure 1

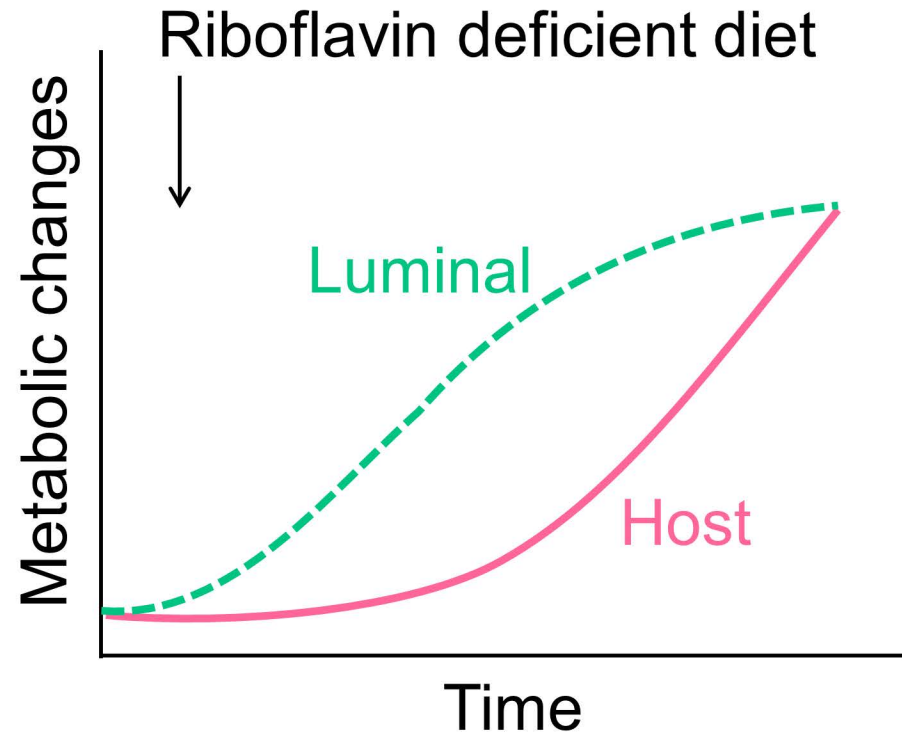
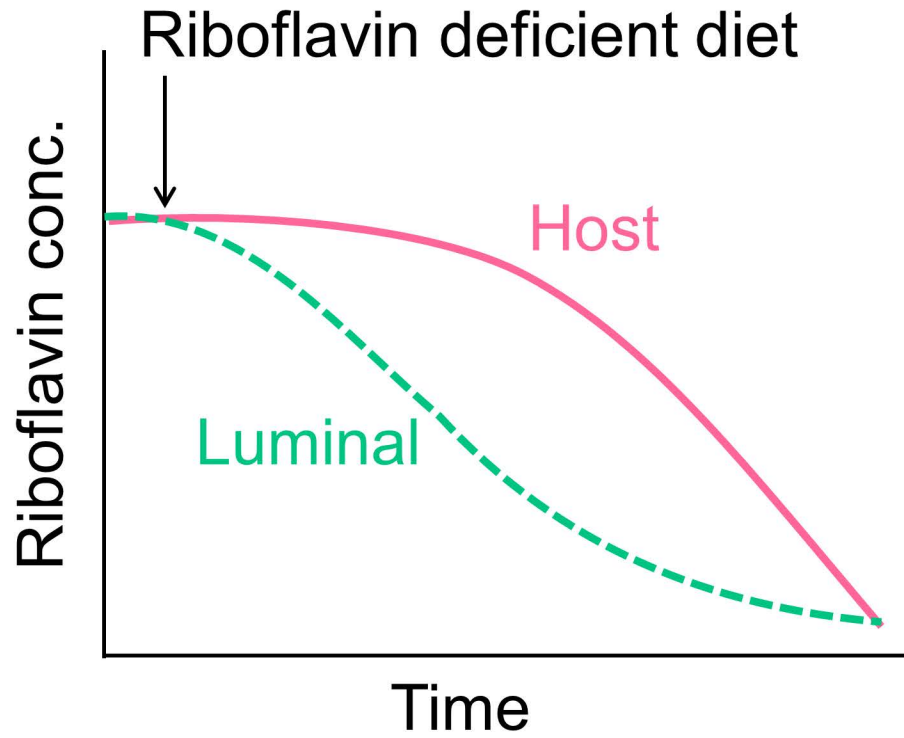
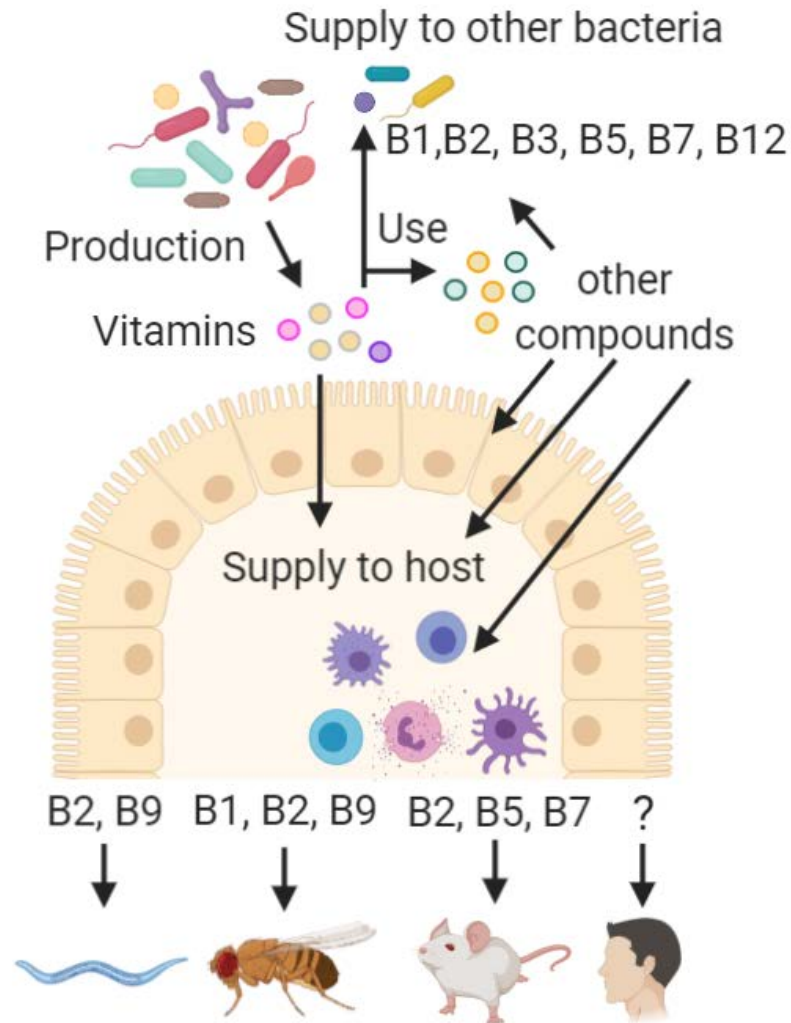


Figure 2



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.