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REVIEW

Vitamin B-12 and the Gastrointestinal Microbiome: A Systematic Review

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ABSTRACT

Vitamin B-12 deficiency is a major public health problem affecting individuals across the lifespan, with known hematological, neurological, and obstetric consequences. Emerging evidence suggests that vitamin B-12 may have an important role in other aspects of human health, including the composition and function of the gastrointestinal (gut) microbiome. Vitamin B-12 is synthesized and utilized by bacteria in the human gut microbiome and is required for over a dozen enzymes in bacteria, compared to only 2 in humans. However, the impact of vitamin B-12 on the gut microbiome has not been established. This systematic review was conducted to examine the evidence that links vitamin B-12 and the gut microbiome. A structured search strategy was used to identify in vitro, animal, and human studies that assessed vitamin B-12 status, dietary intake, or supplementation, and the gut microbiome using culture-independent techniques. A total of 22 studies (3 in vitro, 8 animal, 11 human observational studies) were included. Nineteen studies reported that vitamin B-12 intake, status, or supplementation was associated with gut microbiome outcomes, including beta-diversity, alpha-diversity, relative abundance of bacteria, functional capacity, or short-chain fatty acids (SCFA) production. Evidence suggests that vitamin B-12 may be associated with changes in bacterial abundance. While results from in vitro studies suggest that vitamin B-12 may increase alpha-diversity and shift gut microbiome composition (beta-diversity), findings from animal studies and observational human studies were heterogeneous. Based on evidence from in vitro and animal studies, microbiome outcomes may differ by cobalamin form and co-intervention. To date, few prospective observational studies and no randomized trials have been conducted to examine the effects of vitamin B-12 on the human gut microbiome. The impact of vitamin B-12 on the gut microbiome needs to be elucidated to inform screening and public health interventions. *Adv Nutr* 2022;13:530–558.

Statement of Significance: Vitamin B-12 is synthesized and utilized by bacteria in the human gut microbiome and is required by over a dozen enzymes in bacteria. However, to date, no systematic reviews have been conducted to evaluate the impact of vitamin B-12 on the gut microbiome, or its implications for human health.

Keywords: vitamin B-12, cobalamin, gut microbiota, microbiome, systematic review

Introduction

Vitamin B-12 deficiency is an important public health problem globally (1, 2). Its classic deficiency syndrome is hematological, as pernicious or megaloblastic anemia (2). Inadequate vitamin B-12 status has also been linked to neurological impairments and pregnancy complications (3–5). Emerging evidence suggests that vitamin B-12 may have an important role in other aspects of human health, including modulating the composition and function of the gut microbiome, which may modify the risk of metabolic outcomes and other chronic diseases.

Vitamin B-12 and human health

Vitamin B-12 is a required cofactor for 2 enzymes in humans: L-methyl-malonyl-CoA mutase in the mitochondria and

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Abbreviations used: ACE, abundance-based coverage estimator; MMA, methylmalonic acid; OTU, operational taxonomic unit; PCA, principal components analysis; PCoA, principal coordinates analysis; rRNA, ribosomal RNA; SAM, *S*-adenosylmethionine.

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methionine synthase in the cytoplasm (2). In the mitochondria, adenosylcobalamin is required for isomerization of methyl-malonyl-CoA to succinyl-CoA and is involved in branched-chain amino acid and odd-chain fatty acid catabolism. Methylcobalamin is required for methionine synthase to convert 5-methyl-tetrahydrofolate to tetrahydrofolate for subsequent purine synthesis, and to remethylate homocysteine to methionine for production of *S*-adenosylmethionine (SAM) (6), a methyl donor involved in over 100 methylation reactions (7). Vitamin B-12 is required for DNA synthesis and methylation and folate metabolism; vitamin B-12 deficiency can lead to impairments in cell division, erythropoiesis, DNA stability, and neurological function (3, 4).

Vitamin B-12 is synthesized exclusively by bacteria and is obtained in the diet through consumption of animal-source foods (1, 8). In addition to inadequate dietary intake, vitamin B-12 deficiency can result from low bioavailability or impaired absorption, due to pernicious anemia (an autoimmune disease affecting parietal cells and release of intrinsic factor, required for vitamin B-12 absorption); atrophic gastritis, malabsorption, and risk of pernicious anemia, which increase with age; medications (e.g., proton pump inhibitors); and gastrointestinal diseases (e.g., inflammatory bowel disease) or gastrointestinal infections (e.g., *Helicobacter pylori*, intestinal helminths) (2, 4, 8, 9). Studies in humans suggest that bacterial overgrowth in the small intestines [i.e., predominantly Gram-negative colonic bacteria (10)] can lead to vitamin B-12 deficiency, likely via competition and malabsorption of available vitamin B-12 (11–13).

Vitamin B-12 and the gastrointestinal microbiome

Bacteria in the human gastrointestinal (gut) microbiome synthesize vitamin B-12 and utilize unabsorbed vitamin B-12 from the host (14); however, the impact of vitamin B-12 on the gut microbiome remains largely unexplored. Humans absorb ∼50% of vitamin B-12 at a 1-μg oral dose in the ileum (lower part of the small intestine), and absorption decreases with increasing dose (15). Vitamin B-12 not absorbed in the ileum can reach the large intestine, where gut bacteria metabolize and convert ∼80% of vitamin B-12 into vitamin B-12 analogs (i.e., cobamides with no known vitamin B-12 activity) by altering the benzimidazole base of vitamin B-12 (12, 16). The ability to utilize vitamin B-12 (and vitamin B-12 analogs) may be a competitive advantage for certain bacteria (14, 17, 18). Further, the gut microbiota composition and function may differ in environments that are replete in vitamin B-12 compared with those with inadequate vitamin B-12.

Vitamin B-12 is required for over a dozen enzymes in bacteria (e.g., methyltransferases, isomerases), but only 2 enzymes in mammals. Vitamin B-12 also regulates bacterial genes through riboswitches and other mechanisms (14). Additionally, the majority of gut bacteria have genes encoding transporters for vitamin B-12 and its analogs. Degnan et al. (18) demonstrated that loss-of-function mutations of the genes for these transporters reduced the abundance and competitive ability in the gut of the corresponding strains.

Research gap

Vitamin B-12 is synthesized and utilized by bacteria in the human gut microbiome and is required by over a dozen enzymes in bacteria. Narrative reviews have examined the potential role of vitamin B-12 in human gut microbiome composition and function, mostly through evidence from model bacteria (14, 19, 20). However, to date, no systematic reviews have been conducted to evaluate the effects of vitamin B-12 on the gut microbiome, or its implications for human health.

The objective of this systematic review was to examine the evidence that links vitamin B-12 and the gut microbiome. We evaluated evidence from in vitro, animal, and human studies on the impact of vitamin B-12 on gut microbiome composition and function.

Methods

Search strategy

A structured literature search was conducted using the MEDLINE database via PubMed on 28 March 2020 and updated on 26 March 2021. The following search strategy, including terms for vitamin B-12 and the gut microbiome, was used: (Vitamin B 12 [MeSH] OR Vitamin B 12 Deficiency [MeSH] OR Anemia, Macrocytic [MeSH] OR (Vitamin [all fields] AND (B12 [all fields] OR B 12 [all fields] OR B-12 [all fields])) OR cobalamin* [all fields] OR transcobalamin* [all fields] OR cyanocobalamin* [all fields] OR methylcobalamin* [all fields] OR methylmalonic acid* [all fields] OR hydroxycobalamin* [all fields] OR holotranscobalamin* [all fields] OR holo-transcobalamin* [all fields] OR holo transcobalamin* [all fields] OR ((pernicious [all fields] OR macrocytic [all fields] OR megaloblastic [all fields]) AND (anemia [all fields] OR anaemia [all fields])))) AND (microbiota [MeSH] OR microbiota [all fields] OR microbiom* [all fields] OR ((Gastrointestinal [all fields] OR Gastro-intestinal [all fields] OR Gut [all fields] OR Intestin* [all fields] OR Fecal [all fields] OR colon* [all fields]) AND (flora [all fields] OR microflora [all fields])))). This search strategy was translated and performed in CINAHL, Scopus, Web of Science, Biosis Review, and CABI (CAB Abstracts and Global Health). No date or language filters were applied. The protocol for this review was registered in PROSPERO, the international prospective register of systematic reviews of the University of York and the National Institute for Health Research, under the number CRD42020163772 (21).

Study design

Randomized trials (randomization at the individual or cluster level), quasi-randomized trials (randomization by another method, e.g., alternate allocation), nonrandomized trials, and observational studies were eligible for inclusion.

We also included data from studies using in vitro (i.e., gut simulators) and animal models.

Participants

Studies of interventions in participants with critical illnesses or severe comorbidities, including conditions affecting vitamin B-12 metabolism or the gut microbiome, were excluded. Data from animal models with induced colitis were excluded. There were no other restrictions on participants or population. Studies were also eligible for inclusion if the exposure (vitamin B-12) and outcome (gut microbiome composition/function) were evaluated in mother–infant pairs, respectively.

Exposure: vitamin B-12

Vitamin B-12 as an exposure included vitamin B-12 supplementation (tablet, capsule, dispersible tablet), fortification (any food vehicle), intramuscular/intravenous injection, vitamin B-12 status [i.e., serum/plasma total vitamin B-12, methylmalonic acid (MMA), holotranscobalamin], and dietary intake. We also considered genetic polymorphisms in host genes involved in vitamin B-12 metabolism (e.g., rs9473555 polymorphism in methylmalonyl-CoA mutase). Vitamin B-12 interventions were not restricted by dose, frequency, formulation, or form of cobalamin (e.g., cyanocobalamin, methylcobalamin). Studies with co-interventions were eligible for inclusion, provided they were the same in both the intervention and control groups.

Outcomes: gut microbiome

Primary outcomes for gut microbiota composition included: *1*) beta-diversity (i.e., differences among microbiome samples or groups of samples), *2*) alpha-diversity (i.e., diversity within a microbiome sample), and *3*) relative abundance or concentration of bacteria. Secondary outcomes included *1*) functional capacity, *2*) gut-derived metabolites, and *3*) other biomarkers of gut health. Data on gut microbiome composition or functional capacity were considered if determined by culture-independent methods [e.g., shotgun sequencing, 16S ribosomal RNA (rRNA) gene sequencing].

Study selection

References captured by the search strategy were screened using Covidence (Veritas Health Innovation, Melbourne, Australia; www.covidence.org). Titles and abstracts were independently screened by 2 reviewers (HMG and SLH/AMF). Full texts were retrieved for potentially eligible studies and independently assessed for inclusion by 2 reviewers. Discrepancies were resolved through discussion with the senior author.

Data extraction and management

Using a piloted data extraction form, 2 authors (HMG and SLH/AMF) independently extracted the following data from figures, tables, text, and supplementary materials of included studies: author's last name, publication year, study design, setting and study population or experimental in vitro/animal model, vitamin B-12 intervention or exposure, laboratory methods for gut microbiome assessment (i.e., sample collection, DNA extraction, sequencing techniques, microbial gene region amplification, bioinformatics), and results reported at any time point using any analysis method. Vitamin B-12 concentrations were converted from picograms/milliliter to picomoles/liter (by a factor of 0.7378), for interpretation and comparability of findings. Study authors were contacted via e-mail to clarify population characteristics, methods, or results, as needed. We planned a priori to conduct meta-analyses, subgroup analyses (i.e., by vitamin B-12 status; dose, frequency, and duration of vitamin B-12 intervention; co-interventions), and risk-of-bias assessment for randomized trials, quasi-randomized trials, and nonrandomized trials. Studies were summarized in narrative form and in tables.

Results

Literature review

The structured literature search resulted in 1939 records. After 797 duplicates were removed, 1142 records were reviewed for potential inclusion in the review; and after 966 records were excluded, 176 full-text articles were extracted for review. Of these studies, 154 studies were excluded: $n = 11$ reviews, $n = 4$ duplicates, $n = 2$ in clinical populations, $n = 51$ no vitamin B-12 data, $n = 15$ no gut microbiome data, $n = 52$ for ineligible laboratory methods (i.e., not using culture-independent sequencing techniques), and $n = 19$ did not report associations between vitamin B-12 and gut microbiome outcomes. A total of 22 studies were included in the review: 3 in vitro, 8 animal, and 11 observational human studies. The structured search is summarized in **Figure 1**.

Characteristics of included studies

Characteristics of included in vitro, animal, and human studies are summarized in **Tables 1–3**, respectively.

In vitro studies.

The 3 studies (22–24) using in vitro models provided vitamin B-12 supplementation: 8 treatment groups administered 0.5 mM of vitamin B-12 (cyanocobalamin or adenosylcobalamin) and/or whey protein (beta-lactoglobulin or alpha-lactalbumin) (22), methylcobalamin or cyanocobalamin (i.e., 300 mL of 1.25 mg/L) compared with a control group (23), or cyanocobalamin supplementation alone as 1 of 2 doses or provided in cyanocobalamin-enriched spinach (0.94 mg/g spinach or 0.78 mg/g spinach) compared with a control group (24). Studies providing co-interventions reported that whey protein increased the stability of cyanocobalamin and adenosylcobalamin by 2.2% and 19.7%, respectively (22), and spinach increased cyanocobalamin stability by 5% (24).

Animal studies.

Eight of the included laboratory studies were conducted in animal models, including a wide range of organisms with different microbiomes: 5 in rodents (25–29), 2 in geese

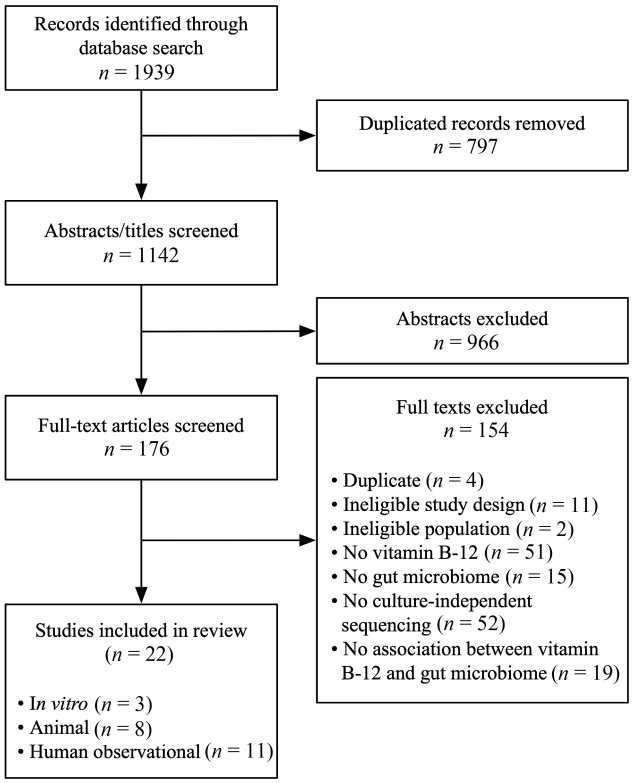

FIGURE 1 Flowchart of the search strategy and results of screening.

(30, 31), and 1 in shrimp (32). In studies with rodent models, vitamin B-12 supplementation was administered as cyanocobalamin (3.94 μg/mL) (25); cyanocobalamin at a 0-mg/kg (deficient), 50-mg/kg (sufficient), or 200-mg/kg dose (27); cyanocobalamin or methylcobalamin at a high dose (1.25 mg/L) (26); or cobalamin (unspecified form, 25 μg/kg diet) provided with or without folic acid (28). One study in rats evaluated serum vitamin B-12 concentrations as the exposure (29). The vitamin B-12 content of diets provided to the control group in rodent studies varied between studies, and study authors provided a small amount of vitamin B-12 (i.e., 0.08 mg/kg cyanocobalamin) (25), a diet with ethanol-washed casein to reduce background vitamin B-12 (27), no vitamin B-12 (28), or did not specify (26, 29). In studies in geese, vitamin B-12 supplementation was administered as low (0.009 mg/kg), medium (0.018 mg/kg), or high (0.036 mg/kg) doses in combination with folic acid (at 0.55 or 2.50 mg/kg) in a 2 × 3 factorial design (30) or as 6 different doses (0.0, 0.005, 0.010, 0.015, 0.020, or 0.025 mg/kg) (31). In the study conducted in shrimp, MMA was assessed in hemolymph (fluid equivalent to blood in invertebrates) (32).

Observational studies.

All 11 studies conducted in human populations were observational, including 6 cross-sectional studies (33–38); 4 cohort studies with durations of 2 (39), 3 (40, 41) or 9 (42) mo; and 1 publication that reported results from a cross-sectional analysis at baseline and a 4-wk intervention study without a control group (43). Nine of the 11 observational studies evaluated dietary intake of vitamin B-12 using 24-h recalls (33, 36, 40), 3-d food records (38), or food-frequency questionnaires (34, 35, 37, 41, 42). Total serum vitamin B-12 concentrations were evaluated in 2 studies (39, 43). Boran et al. (43) also administered intramuscular vitamin B-12 (hydroxycobalamin) injections in a subset of infants with vitamin B-12 deficiency, as part of an intervention study without a control group.

Study populations included infants (1 study; $n = 88$, 4–6 mo) (43); children (1 study; $n = 75$, 2–9 y) (36); mother–infant pairs (2 studies; $n = 22$ and $n = 73$ dyads) (37, 41); women of reproductive age who were lactating (33) or not pregnant or lactating (38, 40) (3 studies; $n = 20$ to 102, 18–40 y), and adults over 50 y of age (35), over 65 y of age (34, 39), or with an unknown age (42) (4 studies; $n = 35$ to 69).

Qualitative synthesis of evidence from in vitro studies

Findings from the 3 included in vitro studies are summarized in **Table 4** (primary outcomes) and **Table 5** (secondary outcomes).

Alpha-diversity.

Findings from in vitro studies suggest that vitamin B-12 supplementation may increase alpha-diversity, and results varied by the form and dose of cobalamin administered and co-interventions. In 1 study (22), adenosylcobalamin resulted in increased alpha-diversity compared with cyanocobalamin, but lower diversity when both were combined with alpha-lactalbumin. Cyanocobalamin or adenosylcobalamin in combination with whey proteins resulted in increased alpha-diversity compared with beta-lactoglobulin alone, but not alpha-lactalbumin alone. In a different study, methylcobalamin resulted in lower alpha-diversity based on Chao1 (measurement of richness) but higher diversity based on Shannon index (measurement of richness and evenness), compared with cyanocobalamin and control groups (23). The cyanocobalamin group had a similar Chao1 index compared with the control group, but higher diversity based on the Shannon index compared with the control group. In the study where cyanocobalamin was administered in a supplement or in spinach (24), alpha-diversity increased in the group receiving the low dose of cyanocobalamin-enriched spinach, did not change in the control group, and decreased in all of the other groups (24).

Beta-diversity.

Three in vitro studies (22–24) reported changes in beta-diversity: findings indicated that vitamin B-12 shifted microbiome composition. In the aforementioned study of whey protein (22), although principal component analyses (PCA) did not provide relevant data for this review, based on hierarchical clustering, groups receiving alpha-lactalbumin alone and cyanocobalamin in combination with beta-lactoglobulin appeared to cluster together. In another study (23), the cyanocobalamin and control groups were clustered more

TABLE 1 In vitro studies assessing the association between vitamin B-12 and the gut microbiome[1]

First author, year (ref)	In vitro model	Vitamin B12 intervention	Specimen	DNA extraction	Sequencing	Multiple comparison method
Wang, 2019 (22)	In vitro colon fermentation using stool samples from a 3-y-old and a rice starch–based nutritive medium	8 groups receiving 0.5 uM/d of 1 of the following for 10 d: whey protein alone (BL, AL), B12 alone (AC, CC), or whey protein with B12 (AC + BL, CC + BL, AC + AL, CC + AL)	Fermentation broth (daily)	MicroElute Genomic DNA kit (Omega Inc.)	V3–V4 16S rRNA region, Illumina MiSeq	Duncan's multiple comparison test
Xu, 2018 (23)	In vitro colon fermentation using stool samples from 5 vitamin B-12–deficient patients and a corn starch–based nutritive medium	3 groups receiving 1 of the following for 7 days: MC (1.25 mg/L), CC (1.25 mg/L), control (no supplementation)	Fermentation broth (day 4 and 7)	Genomic DNA kit (Qiagen)	V3–V4 16S rRNA region, Illumina MiSeq	Duncan's multiple comparison test
Zheng, 2021 (24)	In vitro colon fermentation using stool samples from 3 individuals aged 20–25 y and a nutritive medium (starch type not specified)	5 groups receiving 1 of the following for 3 days: CC supplementation at a high or low dose, or CC-enriched spinach at a high (0.94 ug CC/g spinach) or low (0.78 μg CC/g spinach) dose, compared with control (no supplementation)	Fermentation broth (day 0 and 2)	Genomic DNA kit DP320 (Tiangen)	V3–V4 16S rRNA region, PCR	Duncan's multiple comparison test

[1] AC, adenosylcobalamin; AL, alpha-lactalbumin; B12, vitamin B-12; BL, beta-lactoglobulin; CC, cyanocobalamin; MC, methylcobalamin; ref, reference; rRNA, ribosomal RNA; V, hypervariable region.

closely than the group that received methylcobalamin, based on hierarchical clustering. In this same study based on PCA plots (23), treatment groups appeared distinct from one another and between days 4 and 7 within each group. In another study (24), groups receiving low-dose cyanocobalamin supplementation and high-dose cyanocobalamin-enriched spinach appeared to cluster further from baseline and from the other groups, based on PCA plots.

Bacterial abundance.

The 3 in vitro studies reported associations between vitamin B-12 supplementation and the relative abundance of bacteria. At the phylum level, adenosylcobalamin or cyanocobalamin supplementation alone resulted in higher Bacteroidetes:Firmicutes ratios compared with most other groups receiving whey protein alone or in combination with vitamin B-12, but the 2 forms of vitamin B-12 were not significantly different from one another (22). The ratio of Bacteroidetes:Firmicutes in the groups receiving cyanocobalamin or adenosylcobalamin in combination with whey protein did not differ from each other or from whey protein alone. Another study (24) reported within-group changes in the relative abundance of 3 major phyla; in most groups, cyanocobalamin supplementation or cyanocobalamin-enriched spinach increased Proteobacteria and decreased Firmicutes and Bacteroidetes. However, Proteobacteria decreased and Firmicutes did not change in the low-dose cyanocobalamin-enriched spinach group and Bacteroidetes did not change in the high-dose cyanocobalamin-enriched spinach group.

Bacteroidetes phylum. The relative abundances of the order Bacteroidales and genus *Bacteroides* were differentially enriched in the control group compared with methylcobalamin and cyanocobalamin groups (23). Similarly, cyanocobalamin and adenosylcobalamin in combination with beta-lactoglobulin resulted in a lower abundance of *Bacteroidaceae* compared with beta-lactoglobulin alone (22). However, cyanocobalamin in combination with alpha-lactalbumin resulted in a higher relative abundance of *Bacteroidaceae* compared to alpha-lactalbumin alone and compared to adenosylcobalamin with alpha-lactalbumin;

TABLE 2 Animal studies assessing the association between vitamin B-12 and the gut microbiome[1]

First author, year (ref)	Animal model	*n*	B12 intervention or exposure	Specimen	DNA extraction	Sequencing	Multiple comparison method
Murine models							
Kelly, 2019 (25)	C57BL/6 mice age 8–16 wk	24 ($n = 12$ baseline, $n = 12$ endline)	2 groups receiving CC in drinking water (3.94 μg/mL) for 16 d or a control group	Fecal pellets and cecum contents	Power Fecal DNA Isolation Kit (MO BIO Laboratories)	V3–V4 16S rRNA region, Illumina MiSeq platform	Tukey's and Dunnett's multiple comparison test and Bonferroni correction
Lurz, 2020 (27)	C57BL/6 mice age 3 wk, with induced acute colitis [via 5% (wt:vol) DSS] after day 28	24	3 noncolitis groups receiving the following diets for 28 d: deficient (0 mg/kg), medium (50 mg/kg), or high (200 mg/kg) dose of CC	Fecal pellets	FastDNA spin kit for soil (MP Biomedicals)	V3 16S rRNA region, Ilumina MiSeq platform	FDR
Park, 2019 (28)	Male Sprague Dawley rats, with amyloid-β infused in hippocampus, a cannula inserted in hippocampus, being fed a high-fat diet	50	5 groups receiving the following diets for 8 wk: control diet (25 μg/kg B12 and 2.5 mg/kg folate), B12 deficient (2.5 mg/kg folate), folate deficient (25 μg/kg B12), folate and B12 and folate deficient (no supplementation), sham operated on the control diet	Fecal pellets	Power Water DNA Isolation Kit (Qiagen)	Metagenome, Illumina MiSeq platform	Tukey's multiple comparison test
Siddharth, 2017 (29)	Wistar rats, 8–24 mo old: 8 mo (adults), 18 mo (pre-sarcopenic), and 24 mo (sarcopenic)	30	Serum B12 concentrations	Fecal pellets	PowerMag DNA extraction kit (MO BIO)	V4–V6 rRNA region, Illumina MiSeq platform	FDR

(*Continued*)

TABLE 2 (*Continued*)

First author, year (ref)	Animal model	*n*	B12 intervention or exposure	Specimen	DNA extraction	Sequencing	Multiple comparison method
Zhu, 2019 (26)	Male C57BL/6 mice (10 wk old) with and without induced acute colitis [via 5% (wt:vol) DSS]	36 without colitis ($n = 6$ per group)	3 noncolitis groups receiving the following in drinking water for 3 d: CC (1.25 mg/L), MC (1.25 mg/L), control (no supplementation)	Feces washed out from colon with saline	MicroElute Genomic DNA kit (Omega Bio-tek, Inc.)	V3–V4 16S rRNA region, Illumina MiSeq	Duncan's multiple range test
Non–murine models							
Cheng, 2018 (30)	Wulong geese, 1-day-old	72 ($n = 2$ per replicate, 6 replicates)	2 × 3 factorial design with groups receiving either low (0.55 mg/kg) or high (2.50 mg/kg) dose of folic acid, and either low (0.009 mg/kg), medium (0.018 mg/kg), or high (0.036 mg/kg) dose of B12 added to feed for 4 wk	Cecum contents	Genomic DNA kit (Tiangen Biochemical Technology Co., Ltd.)	V3–V4 16S rRNA region, PCR	Not specified
Long, 2018 (31)	Wulong geese, 4 weeks old	72 ($n = 2$ per replicate, 6 replicates)	6 groups receiving the following doses of B12 for 11 wk: *1*) 0.000 mg/kg, *2*) 0.005 mg/kg, *3*) 0.010 mg/kg, *4*) 0.015 mg/kg, *5*) 0.020 mg/kg, *6*) 0.025 mg/kg	Cecum contents	Genomic DNA kit (Tiangen Biochemical Technology Co., Ltd.)	V3–V4 16S rRNA region, PCR	Not specified
Duan, 2021 (32)	Healthy Litopenaeus vannamei (Pacific white shrimp); 4 groups receiving various stress tests (i.e., thermal stress, ammonia stress, ammonia and thermal stress)	200	Methylmalonic acid concentrations in hemolymph (i.e., invertebrate equivalent of blood)	Intestines without feces	TAB/SDS method	V4 16S rRNA region, PCR	Tukey's multiple comparison test

[1] B12, vitamin B-12; CC, cyanocobalamin; DSS, dextran sulfate soldium; FDR, false discovery rate; MC, methylcobalamin; ref, reference; rRNA, ribosomal RNA; TAB/SDS, trimethyl ammonium bromide/sodium dodecyl sulphate; V, hypervariable region.

TABLE 3 Human studies assessing the association between vitamin B-12 and the gut microbiome[1]

First author, year (ref)	Country	Study design (duration, if applicable)	Participants	*n*	Sex, % female	BMI,[2] kg/m^2	B12 exposure	Baseline B12 status or intake[2]	Specimen	DNA extraction	Sequencing	Multiple comparison method
Boran, 2020 (43)	Turkey	Cross-sectional	4–6-mo-old healthy term, exclusively breastfed infants (53.4% vaginal delivery), with ($n = 60$) and without ($n = 28$) B12 deficiency (<150 pmol/L)	88	50	Not reported; ≥2500 g[3]	Serum total B12	119.5 (93.0, 162.8) pmol/L	Stool	DNA extraction: QuickGene (Kurabo, Osaka, Japan)	16S rRNA V4 region, Illumina MiSeq	FDR
		Uncontrolled before and after (4 wk)	4–6-mo-old healthy term, exclusively breastfed infants with B12 deficiency (<150 pmol/L)	11	Not reported	Not reported; ≥2500 g[3]	Intramuscular HC, 250—500 μg 2× weekly (1 wk) then 500 μg weekly (3 wk)	Before ($n = 60$): 100.7 (81.9, 121.4); after ($n = 11$): 770.3 (360.0, 1106.7) pmol/L				
Babakobi, 2020 (41)	Israel	Cohort (3 mo)	Healthy women (25–40 y) who gave birth vaginally, and their infants born at term, recruited 1–2 d postpartum	22	Infants: 64	23.3 ± 4.6 (range: 19–37)	B12 intake, FFQ, past 12 mo	Not reported	Infant stool swab	PowerSoil DNA extraction kit (MO BIO)	16S rRNA V3–V4 region, Illumina MiSeq	FDR
Carrothers, 2015 (40)	USA	Cohort (3 mo)	Lactating women (self-reported breastfeeding; 25–37 y)	20	100	23.5 ± 3.3 (range: 19.3–34.5)[4]	B12 intake, 24HR (excluded supplements)	4.7 ± 3.1 (range: 1.3–13.1) μg/d	Stool	QIAamp DNA Stool Mini Kit (Qiagen)	16S rRNA V1–V3 region, Illumina MiSeq	Not reported
Gurwara, 2019 (35)	USA	Cross-sectional	Male veterans (50–75 y), with an endoscopically normal colon after an elective colonoscopy procedure July 2013–April 2016	35	0	33.6 ± 6.5 and 33.9 ± 6.6[3] ($n = 97$)	B12 intake, FFQ, past 12 mo	2.38 (1.73–2.99) μg/d	Colonic mucosal samples	MO BIO PowerLyzer UltraClean Tissue & Cell DNA Isolation Kits (MO BIO Laboratories)	16S rRNA V4 region, Illumina MiSeq platform	FDR

(*Continued*)

TABLE 3 (*Continued*)

First author, year (ref)	Country	Study design (duration, if applicable)	Participants	*n*	Sex, % female	BMI,[2] kg/m^2	B12 exposure	Baseline B12 status or intake[2]	Specimen	DNA extraction	Sequencing	Multiple comparison method
Herman, 2020 (36)	USA	Cross-sectional	Healthy children 2–9 y (not breastfeeding, ~84% ever breastfed; $n = 30$, 2–3 y; $n = 45$, 4–9 y)	75	2–3 y: 57; 4–9 y: 62	2–3 y: 30% ow/ob, 3% uw; 4–9 y: 31% ow/ob, 9% uw	B12 intake, three 24HRs (included supplements)	2–3 y: 4.31 ± 1.78; 4–9 y: 5.6 ± 7.36 μg/d	Stool swab	MO BIO PowerSoil isolation kit (MO BIO)	16S rRNA V4 region, Illumina MiSeq platform	FDR
Mörkl, 2018 (33)	Austria	Cross-sectional	Females (18–40 y; 24.6 ± 4.6 y), not pregnant or lactating, including patients with anorexia nervosa ($n = 17$), athletes ($n = 20$), and women with normal ($n = 25$), overweight ($n = 21$), or obese ($n = 19$) BMI	102	100	24.28 ± 6.5	B12 intake, two 24HRs	3.19 ± 1.58 and 4.14 ± 2.13 μg/d	Stool	PowerLyzer PowerSoil DNA Isolation Kit (MO BIO)	16S rRNA V1–V2 region, Ion Torrent PGM using the Ion 400BP Sequencing Kit	Not reported
Selma-Royo, 2021 (37)	Spain	Nested cross-sectional	Mother-infant dyads at delivery with a healthy pregnancy; 62% vaginal delivery; mothers were ≥18 y [35 (31.0, 36.25) y]	73	Infants: 45	21.8 (20.43, 24.13)[4] 3323 ± 416 g[3]	Maternal B12 intake, FFQ	10 ± 8 μg/d	Maternal and infant stool swabs	Master-Pure DNA extraction kit (Epicentre)	16S rRNA V3–V4 region, Illumina MiSeq	FDR
Seura, 2017 (38)	Japan	Cross-sectional	Healthy, normal weight women, 20–22 y	28	100	Not reported	B12 intake, 3 food records (excluded supplements)	Not reported	Stool collected in guanidine thiocyanate	Not reported	T-RFLP method	Not reported

(*Continued*)

TABLE 3 (*Continued*)

First author, year (ref)	Country	Study design (duration, if applicable)	Participants	*n*	Sex, % female	BMI,[2] kg/m^2	B12 exposure	Baseline B12 status or intake[2]	Specimen	DNA extraction	Sequencing	Multiple comparison method
Shah, 2017 (42)	Not specified	Cohort (9 mo)	Healthy participants	9	Not reported	Not reported	B12 intake, NIH Dietary History Questionnaire	Not reported	Stool and colonic mucosal sample	Not reported	16S rRNA, 454 Roche Titanium	Not reported
Tamura, 2017 (34)	Japan	Cross-sectional	Healthy older adults (range: 65–84 y; 72.1 ± 0.6 y)	56	45	23.1 ± 0.4	B12 intake, FFQ, past 1 wk	6.83 ± 0.33 μg/d	Stool	Magtration System 12GC and GC series MagDEA DNA 200 reaction cartridge (Precision System Science)	16S rRNA, Illumina MiSeq	Not reported
Valentini, 2015 (39)	France, Germany, Italy	Cohort within randomized trial (8 wk)	Healthy older adults (65–85 y; 70.1 ± 3.9 y), with BMI 22–30 kg/m^2 and ECOG 0–2	69	51	26.8 ± 3.59	Total serum B12	297.3 ± 20.0 and 307.7 ± 28.1 pmol/L	Stool	QIAamp DNA Stool Kit (Qiagen)[5]	16S rDNA gene targeted qPCR for Clostridium cluster IV and Bifidobacteria	Not reported

[1] B12, vitamin B-12; ECOG, Eastern Cooperative Oncology Group Performance Status; FDR, false discovery rate; FFQ, food-frequency questionnaire, HC, hydroxycobalamin; ob, obese; ow, overweight; PGM, Personal Genome Machine; ref, reference; rRNA, ribosomal RNA; T-RFLP, terminal restriction fragment length polymorphism; uw, underweight; 24HR, 24-h dietary recall.
[2] Numbers are presented as either median (IQR) or mean ± SD.
[3] Infant birth weight.
[4] Prepregnancy BMI.
[5] Provided by study authors via e-mail correspondence.

TABLE 4 In vitro studies assessing the association between vitamin B-12 and the gut microbiome[1]

First author, year (ref)	Comparison	Alpha-diversity	Beta-diversity	Bacteria abundance
Wang, 2019 (22)	Whey protein with CC or AC (AC + BL, CC + BL, AC + AL, CC + AL) vs. whey protein alone (AL, BL)	AC + BL and CC + BL higher than BL alone AC + AL and CC + AL did not differ from AL alone	AL[2] and CC + BL clustered from others	Bacteroidetes:Firmicutes ratio: not significant Family[2]: CC + BL vs. BL higher *Christensenellaceae*, *Prevotellaceae*, *Cariobacteriacese*, *Clostridiaceae*, *Chloroplast*, *Lactobacillaceae*, *Enterococcaceae*, *Staphylococcaeae*; lower *Bacteroidaceae*, *Eubacteriaceae*, *Rikenellaceae*, *Porphyromonadaceae*, *Verrucomicrobiaceae* Family[2]: CC + AL vs. AL higher *Enterococcaceae*, *Anearoplasmataceae*, *Bacteroidaceae*; lower *Peptostreptococcaceae*, *Prevotellaceae*, *Coriobacteriaceae*, *Clostridiaceae*, *Streptococcaceae*, *Mogibacteriaceae*, *Enterobacteriaceae* Family[2]: AC + BL vs. BL higher *Erysipelotrichaceae*, *Enterobacteriaceae*; lower *Lachnospiraceae*, *Bacteroidaceae*, *Eubacteriaceae*, *Rikensellaceae*, *Porphyromonadaceae*, *Verrucomibrobiaceae* Family[2]: AC + AL vs. AL higher *Leuconostocaceae*; lower *Peptostreptococcaceae*, *Christensenellaceae*, *Prevotellaceae*, *Coriobacteriaceae*, *Clostridiaceae*, *Streptococcaceae*, *Mogibacteriaceae*, *Enterobacteriaceae*
	CC vs. AC	CC had lower diversity compared with AC		Bacteroidetes:Firmicutes ratio: not significant Family[2]: Higher *Bacteroidiaceae*, *Eubacteriaceae*; lower *Bifidobacteriaceae*, *Flavinobacteriaceae*, *Peptostreptococcaceae*, *Lactobacillaceae*, *Ruminococcaceae*
	CC + AL vs. AC + AL	CC + AL had higher diversity compared to AC + AL		Bacteroidetes:Firmicutes ratio: not significant Family[2]: Higher *Christensenellaceae*, *Enterococcaceae*, *Lachnospiraceae*, *Anaeroplasmataceae*, *Bacteroidiaceae*; lower *Leuconostocaceae*
	CC + BL vs. AC + BL	Not significant		Bacteroidetes:Firmicutes ratio: not significant Family[2]: Higher *Prevotellaceae*, *Cariobacteriacese*, *Clostridiaceae*, *Chloroplast*, *Staphylococcaeae*, *Lactobacillaceae*, *Enterococcaceae*, *Lachnospiraceae*; lower *Enterobacteriaceae*

(*Continued*)

TABLE 4 (*Continued*)

First author, year (ref)	Comparison	Alpha-diversity	Beta-diversity	Bacteria abundance
Xu, 2018 (23)	CC vs. MC vs. control	CC vs. control: lower Shannon index (i.e., higher diversity), Chao1 not significant MC vs. CC and control: lower Chao1 (i.e., lower diversity) and lower Shannon index (i.e., higher diversity)	Control and CC groups clustered[2] Clustering by treatment and day, except MC samples on day 7 were more variable[2]	Genus[2]: MC and CC increased *Acinetobacter* and decreased *Bacteroides*, *Enterobacteriaceae* unc, and *Ruminococcaceae* unc over time Genus: MC was positively correlated with *Pseudomonas* bacteria in canonical correspondence analysis Control group differentially enriched with: -Order: Bifidobacteriales, Bacteroidales, Clostridiales -Family: *Bifidobacteriaceae*, *Bacteroidaceae*, *Rikenellaceae*, *Lachnospiraceae*, *Ruminococcaceae*, *Sutterellaceae* -Genus: *Bifidobacterium*, *Bacteroides*, *Alistipes*, *Dorea*, *Eisenbergiella*, *Lachnospiraceae_unclassified*, *Ruminococcus2*, *Ruminococaccaceae unclassified* CC differentially enriched with: -Order: Fusobacteriales, Caulobacterales -Family: *Fusobacteriaceia*, *Caulobacteraceae* -Genus:*Clostridium* cluster *XlVb*, *Fusobacterium*, *Ruminococcus*, *Brevundimonas* MC differentially enriched with: -Order: Pseudomonadales -Family: *Clostridiacaeae1*, *Clostridiales* family *XI incertae sedis*, *Comamonadaceae*, *Moraxellaceae* -Genus: *Escherichia*, *Rhizobacter*, *Clostridium*, *Sedimentibacter*, and *Flavonifractor*, *Clostridium*, *Eubacteriaceae unclassified*, *Aceinetobacter*, *Moraxellaceae unclassified*
Zheng, 2021 (24)	High- and low-CC supplement (CC-high, CC-low) and CC-enriched spinach (CCspinach-high, CCspinach-low) vs. control		CC-low and CCspinach-high clustered away from baseline and other groups (control, CC-high and CCspinach-low)[2]	Genus[2]: CC-low vs. control: higher *Klebsiella*, *Acinetobaacter*; lower *Comamonas*, *Bacteroides*, *Escherichia-Shigella* CC-high vs. control: higher *Klebsiella*; lower *Bacteroides*, *Escherichia-Shigella*CC-high vs. CC-low: higher *Comamonas*, lower *Acinetobacter*CCspinach-low vs. control: higher *Klebsiella* and *Bacteroides*; lower *Comamonas*CCspinach-high vs. control: higher *Acinetobacter*; lower *Comamonas*, *Klebsiella*, *Escherichia-Shigella*, *Bacteroides*CCspinach-high vs. CCspinach-low: higher *Acinetobacter*; lower *Klebsiella*, *Escheria-Shigella*, *Bacteroides*

(*Continued*)

TABLE 4 (*Continued*)

First author, year (ref)	Comparison	Alpha-diversity	Beta-diversity	Bacteria abundance
	Within-group changes	Alpha-diversity (Shannon, Simpson) decreased in CC-low, CC-high, and CCspinach-high group; increased for the CCspinach-low group; did not change in control group		Phylum: Control: no change in Proteobacteria, Firmicutes, Bacteroidetes CC-low: Higher Proteobacteria, lower Firmicutes and Bacteroidetes CC-high: Higher Proteobacteria, lower Firmicutes and Bacteroidetes CCspinach-low: Lower Proteobacteria and higher Bacteroidetes CCspinach-high: Higher Proteobacteria, lower Firmicutes Genus[2]: Control: higher *Comamonas*, *Klebsiella*; lower *Escherichia-Shigella* CC-low: higher *Acinetobacter*; lower *Escherichia-Shigella* CC-high: higher *Comamonas*, *Klebsiella*; lower *Escherichia-Shigella*, *Bacteroides* CCspinach-low: lower *Escherichia-Shigella*; higher *Bacteroides* CCspinach-high: higher *Acinetobacter*; lower *Escherichia-Shigella*, *Bacteroides*

[1] AC, adenosylcobalamin; AL, alpha-lactalbumin; B12, vitamin B-12; BL, beta-lactoglobulin; CC, cyanocobalamin; MC, methylcobalamin; ref, reference; unc, unclassified.
[2] No statistical test reported, based on study authors or systematic review authors interpretation of figures.

TABLE 5 Studies assessing association between vitamin B-12 and secondary outcomes[1]

First author, year (ref)	Model or population	Comparison	Function	Other outcomes
In vitro studies				
Xu, 2018 (23)	In vitro	CC vs. MC vs. control	Secondary functions: MC and CC promoted lipid, terpenoid, and polyketide metabolism, and degradation of exogenous substances; and inhibited the synthesis of transcription factors and secondary metabolites Tertiary functions: MC and CC promoted pathways for DNA repair and recombinant protein (ko03400) and decreased nitrogen metabolism (ko00910) and starch with sucrose metabolism (ko00500) Tertiary functions: Control group increased ABC transporter (ko02010) pathway	SCFAs: MC was positively correlated with propionate and butyrate in canonical correspondence analysis Enzyme activity[2]: Control had higher protease activity than MC or CC; amylase activity was more stable in control, protease activity fluctuated more in MC, and cellulase activity fluctuated more in CC SCFAs: MC and CC had higher total SCFAs, while control had higher acetate; propionate decreased in control, increased in MC, and did not change in CC; butyrate was highest in MC on day 1 then decreased, and did not change in control or CC
Zheng, 2021 (24)	In vitro	High and low CC supplement (CC-high, CC-low) and CC-enriched spinach (CCspinach-high, CCspinach-low) vs. control; within-group changes	Secondary functions: CC-high: lower glycan biosynthesis and metabolism CCspinach-high: lower enzyme family; higher xenobiotics and metabolism, glycan biosynthesis and metabolism, transport and catabolism, lipid metabolism	SCFAs: CCspinach-low had higher acetate and butyrate vs. other groups
Murine studies				
Kelly, 2019 (25)	C57BL/6 mice (8–16 wk old)	CC vs. control	Not reported	Cecum SCFAs: not significant Transcriptome (IL-1β, IL-6, IL-10, TNF-α, IL-12p70, IFN-γ, mKC): not significant Corrinoid concentrations (cecum): higher cecum cobalamin, MeS-ADE; no difference in cecum Me-ADE or cobinamide
		Post- vs. pre-CC	Not reported	Corrinoid concentrations (stool): lower fecal Me-ADE, MeS-ADE and ADE; higher cobinamide and cobalamin; no difference in control group

(*Continued*)

TABLE 5 (*Continued*)

First author, year (ref)	Model or population	Comparison	Function	Other outcomes
Lurz, 2020 (27)	C57BL/6 mice age 3 wk	CC high (B12++), medium (B12+), and deficient (B12−) dose, and within-group changes	Not reported	Transcriptome (IL-10, TNF-α): not significant
Siddharth, 2017 (29)	Wistar rats	B12 concentrations	Lower K00947 ("none," metabolism) and K02803 ["PTS system, N-acetylglucosamine-specific IIB component (EC:2.7.1.69)," carbohydrate metabolism]	Proteomics: Higher Notch1, complement factor B, thrombospondin-4, IL-1beta, contactin 1, and melanoma inhibitory activity Lower endoplasmic reticulum aminopeptidase 1, alpha S1 casein, chromobox protein homolog 5, proteasome subunit alpha2, nucleoside diphosphate kinase A, IL-17 receptor D, osteoprotegerin ligand/TRANCE, mitochondrial ATP synthase beta-subunit, non-receptor tyrosine kinase c-abl oncogene 1, amnionless, parathyroid hormone, neutral ceramidase, and X-linked ectodysplasin-A2 receptor
Zhu, 2019 (26)	Male C57BL/6 mice (10 wk old)	CC vs. MC vs. control	Not significant	Not reported
Human studies				
Mörkl, 2018 (33)	Females (18–40 y), not pregnant or lactating	B12 intake (continuous, above vs. below median)	Not reported	Higher zonulin concentrations in serum (indicator of gut permeability) with higher continuous B12 intake and above vs. below median, but not significant by tertiles of B12 intake
Tamura, 2017 (34)	Healthy older adults (65–84 y)	B12 intake	Not reported	Lower quercetin degradation, after 7 h incubation of stool samples with quercetin

[1] ADE, adenine cobamide; B12, vitamin B-12; CC, cyanocobalamin; Me-ADE, 2-methyladenine cobamide; MeS-ADE, 2-methylmercaptoadenine cobamide; MC, methylcobalamin; mKC, murine keratinocyte-derived chemokine; ref, reference; TRANCE, tumor necrosis factor-related activation-induced cytokine.
[2] No statistical test reported, based on study authors or systematic review authors interpretation of figures

and cyanocobalamin alone had a higher abundance of *Bacteroidaceae* compared with adenosylcobalamin alone (22), possibly due to differences in cobalamin stability. In another study (24), the genus *Bacteroides* decreased after supplementation with high- or low-dose cyanocobalamin and high-dose cyanocobalamin-enriched spinach, but increased after supplementation with low-dose cyanocobalamin-enriched spinach. The relative abundance of the family *Rikenellaceae* and genus *Alistipes* (both within the Bacteroidales order) were differentially enriched in the control group compared with the groups that received methylcobalamin and cyanocobalamin supplementation (23), and the relative abundance of *Rikenellaceae* was lower in groups receiving cyanocobalamin or adenosylcobalamin in combination with beta-lactoglobulin, compared with beta-lactoglobulin alone (22).

Firmicutes phylum. The relative abundances of the order Clostridiales and family *Ruminococcaceae* were differentially enriched in the control group compared with groups receiving methylcobalamin and cyanocobalamin (23). *Ruminococcaceae* abundance was also lower after cyanocobalamin supplementation alone compared with adenosylcobalamin alone (22). Also within the Clostridiales order, the relative abundance of the family *Clostridiaceae* and genus *Clostridium* were differentially enriched in the methylcobalamin group compared with the cyanocobalamin or control group (23). When compared with whey protein supplementation alone, cyanocobalamin or adenosylcobalamin with alpha-lactalbumin resulted in lower abundance of *Clostridiaceae*, while cyanocobalamin supplementation with beta-lactoglobulin resulted in higher abundance of *Clostridiaceae*, compared with beta-lactoglobulin alone or the combination of adenosylcobalamin with beta-lactoglobulin (22).

Proteobacteria phylum. Within the order Pseudomonadales, the relative abundances of the family *Moraxellaceae* and an unclassified genus in *Moraxellaceae* were differentially enriched in the methylcobalamin group, and the genus *Acinetobacter* (in the family *Moraxellaceae*) was differentially enriched in both the methylcobalamin and adenosylcobalamin groups compared with the control group (23). The relative abundance of *Acinetobacter* was higher in the groups receiving high and low doses of cyanocobalamin supplementation and high-dose cyanocobalamin-enriched spinach compared with the control (24).

The family *Enterobacteriaceae* (order Enterobacterales, phylum Proteobacteria) increased after supplementation with adenosylcobalamin in combination with beta-lactoglobulin compared with beta-lactoglobulin alone or cyanocobalamin in combination with beta-lactoglobulin (22). Adenosylcobalamin or cyanocobalamin supplementation in combination with alpha-lactalbumin resulted in a lower abundance of *Enterobacteriaceae* compared with alpha-lactalbumin alone (22). Within this family, the relative abundance of *Escherichia-Shigella* was differentially enriched in the methylcobalamin supplementation group (23) but decreased after cyanocobalamin supplementation or cyanocobalamin-enriched spinach compared with control (24); and *Klebsiella* increased after cyanocobalamin supplementation or low-dose cyanocobalamin-enriched spinach (24).

Actinobacteria phylum. The control group was differentially enriched in the relative abundance of the order Bifidobacteriales, family *Bifidobacteriaceae*, and genus *Bifidobacteria*, compared with methylcobalamin and cyanocobalamin supplementation (23). In another study, the relative abundance of *Bifidiobacteriaceae* was higher after supplementation with adenosylcobalamin alone compared with cyanocobalamin alone (22).

Bacterial function.

Two of the included in vitro studies (23, 24) reported data for predicted functional outcomes.

Metabolism. Methylcobalamin and cyanocobalamin supplementation (23) and high-dose cyanocobalamin-enriched spinach (24) promoted lipid metabolism pathways and degradation of exogenous substances. High-dose cyanocobalamin supplementation decreased the capacity for glycan biosynthesis and metabolism, while high-dose cyanocobalamin-enriched spinach increased the capacity for this pathway (24). Methylcobalamin and cyanocobalamin groups (23) had a lower capacity for biosynthesis of secondary metabolites and tertiary pathways within carbohydrate (starch and sucrose) and energy (nitrogen) metabolism pathways.

Environmental and genetic information processing. Methylcobalamin and cyanocobalamin supplementation resulted in a lower capacity for the ABC transporter (ko02010) and synthesis of transcription factors and higher capacity for DNA repair and recombinant protein pathway (23).

Cellular processes. The high-dose cyanocobalamin-enriched spinach group promoted pathways for transport and catabolism (24).

Other outcomes. Two of the included studies (23, 24) reported data for other outcomes: short-chain fatty acids (SCFA) concentrations (23, 24) and enzyme activity of protease, amylase, and cellulase (23). In canonical correspondence analysis (23), methylcobalamin was associated with higher propionate and butyrate concentrations. In the other study (24), the butyrate and acetate concentrations decreased in all groups over time, except low-dose cyanocobalamin-enriched spinach increased butyrate over time and ended with higher acetate concentrations compared with other groups, although statistical significance was not reported for these findings.

For enzyme activity (23), the authors reported that the control group had higher protease activity than the

cobalamin groups; protease and cellulase activity had the greatest fluctuations over time in the methylcobalamin and cyanocobalamin groups, respectively, compared with the other groups, while amylase activity was more stable in the control group.

Qualitative synthesis of evidence from animal studies

The results from the 8 included animal studies are summarized in **Table 6** (primary outcomes) and in Table 5 (secondary outcomes).

Alpha-diversity.

Three studies conducted in mice (25–27) and 2 studies in geese (30, 31) reported heterogeneous results for alpha-diversity. In mice, alpha-diversity (Chao1, Shannon index, Simpson index) was not significantly different in the group that received cyanocobalamin compared with a nonsupplemented control group (25). In another study in mice (27), alpha-diversity based on the Chao1 and Shannon indices did not significantly differ by cyanocobalamin dose (0 mg/kg, 50 mg/kg, or 200 mg/kg in feed pellets). In the third study in mice (26), reported alpha-diversity (Chao1, Shannon index) was lower in the cyanocobalamin and methylcobalamin groups compared with the control group, although statistical significance was not reported.

In studies in geese, results from statistical tests were not reported for between-group differences. However, in geese receiving high-dose folic acid, alpha-diversity was U-shaped based on operational taxonomic units (OTUs), abundance-based coverage estimators (ACE), and Chao1 indices, with the lowest diversity in the group receiving the medium vitamin B-12 dose (0.018 mg/kg), while diversity increased from low (0.009 mg/kg) to high (0.036 mg/kg) doses of vitamin B-12, based on Simpson and Shannon indices (30). In geese receiving the low-dose folic acid, diversity decreased for low to high doses of vitamin B-12 for OTU, ACE, and Chao1 indices; diversity was an inverted U-shape based on Simpson and Shannon indices, with the highest alpha-diversity in the group receiving the medium dose of vitamin B-12 (30). In another study in geese, 6 different doses of vitamin B-12 were administered; geese that received the 0.010-mg/kg dose had the highest diversity based on observed OTUs, ACE, and Chao1, while geese in the group that received the 0.020-mg/kg dose had the highest diversity based on Shannon and Simpson indices (31).

Beta-diversity.

Four of the studies in rodents (25–28) and both studies in geese (30, 31) reported beta-diversity, with mixed results. In mice, beta-diversity was significantly different at the genus level before and after cyanocobalamin supplementation, and between the cyanocobalamin and control groups (25). Conversely, beta-diversity did not differ by vitamin B-12 intervention in the other studies of mice (27, 28, 44).

Among geese receiving vitamin B-12 and folic acid (30), in principal coordinates analysis (PCoA) and the unweighted pair group method with arithmetic mean (UPGMA), groups receiving the medium dose of vitamin B-12 appeared to cluster further from groups receiving the low and high doses. In the study evaluating 6 different doses of vitamin B-12 (31), unexpectedly, the group with the lowest vitamin B-12 dose (0 mg/kg) clustered the closest to the group that received the highest dose of vitamin B-12 (0.025 mg/kg), and clustered the furthest from the group that received the second highest dose (0.020 mg/kg).

Bacterial abundance.

All of the included animal studies reported associations between vitamin B-12 supplementation or status and relative abundance of bacteria; however, findings varied by the form of vitamin B-12 and co-interventions. At the phylum level, the relative abundances of Bacteroidetes and Proteobacteria were higher and Firmicutes was lower in mice that received cyanocobalamin compared with methylcobalamin, while both cobalamin supplementation groups had a lower abundance of Bacteroidetes and higher Firmicutes compared with the control group (26). Although a study in rats did not report phylum-level results, classes within the Proteobacteria phylum (Alphaproteobacteria, Gammaproteobacteria) were higher in the vitamin B-12–deficient group compared with the control group (28). Among geese receiving vitamin B-12 and folic acid (30), the relative abundance of Bacteroidetes increased from the lowest to highest doses of vitamin B-12; the relative abundances of Proteobacteria and Firmicutes were U-shaped, with the medium dose of vitamin B-12 (0.018 mg/kg) having the lowest abundance of Proteobacteria and highest abundance of Firmicutes.

Bacteroidetes phylum. Several studies reported the relative abundance of the genus *Bacteroides*. Cyanocobalamin supplementation (3.94 μg/mL and 200 mg/kg) in mice decreased the abundance of *Bacteroides* (25, 27); interestingly, the relative abundance of *Bacteroides* also decreased in the groups receiving low but not medium doses (0 and 50 mg/kg) (27). In geese, *Bacteroides* abundance was higher in groups that received high (0.036 mg/kg) or medium (0.018 mg/kg) vitamin B-12 doses (30). Other genera in the Bacteroidetes phylum include *Prevotella*, which increased in mice that received 0 mg/kg or 50 mg/kg of cyanocobalamin but not the higher dose (200 mg/kg) (27), and *Alistipes*, which was highest in geese that received the highest dose of vitamin B-12, among groups receiving the higher dose of folic acid (30). In shrimp, higher MMA concentrations (i.e., lower vitamin B-12 status) were associated with increased abundance of several genera in the *Flavobacteriaceae* family (32).

Firmicutes phylum. Several families and genera in the Clostridiales order were impacted by vitamin B-12. In mice receiving 3 different doses of cyanocobalamin (0, 50, 200 mg/kg) (27), relative abundance of the genus *Clostridium* (in the *Clostridiaceae* family) increased in groups receiving 0- and 50-mg/kg cyanocobalamin supplementation, but not 200 mg/kg. The relative abundance of the *Lachnospiraceae* family decreased in the medium-dose group (50 mg/kg) but

TABLE 6 Animal studies assessing the association between vitamin B-12 and the gut microbiome[1]

First author, year (ref)	Comparison	Alpha-diversity	Beta-diversity	Relative abundance
Murine models				
Kelly, 2019 (25)	Post- vs. pre- CC supplementation	Chao1, Shannon H, Simpson: not significant	Significant difference at genus level but not phylum level	*Bacteroides* decreased (2.50% baseline, 0.54% endline)
	CC vs. control at endline	Chao1, Shannon H, Simpson: not significant	Significant difference	Not reported
Lurz, 2020 (27)	High (B12++), medium (B12+), and deficient (B12−) dose CC, and within-group changes	Shannon index: not significant Chao1: not significant	No clustering by B12 diet[2]	Genus increased in at least 1 group: increased *Parabacteroides*, *Sutterella*, *Rikenellaceae* unc, *Mogibacteriaceae* unc (all groups), *Bilophila*, *Prevotella*, *Mucispirillium*, *Clostridium* (B12 + and B12++), *Moryella*, *Clostridiaceae* unc (B12+), *Eubacterium* (B12++) Genus decreased in at least 1 group: *Ruminococcus* (all groups), *Bacteroides* (B12- and B12++), RF32 unc, Clostridiales (B12−), *Lachnospiraceae* (B12+)
Park, 2019 (28)	Control diet (B12 and folate) vs. B12 deficient (folate)	Not reported	No clustering by B12 diet[2]	Class: lower Gammaproteobacteria and Alphaproteobacteria Class: higher Deferribacteres and Actinobacteria
Siddharth, 2017 (29)	B12 concentrations	Not reported	Not reported	Genus: higher *Lutispora*, *Adlecreutzia*, *Papillibacter*, *Clostridium* XIVa Genus: lower *Lachnobacterium*, *Clostridium* XIVb, *Hydrogenoanaerobacterium*, *Coprococcus*, *Suterella*
Zhu, 2019 (26)	CC vs. MC vs. control	Chao1, Shannon index: higher in control group[2]	No clustering by B12 supplement[2]	Phylum[2]: MC vs. CC had higher Firmicutes and Proteobacteria and lower Bacteroidetes Phylum[2]: MC and CC vs. control had higher Firmicutes and lower Bacteroidetes Genus[2]: MC had higher Anaerotruncus (vs. others); CC had higher Oscillospira (vs. others); MC and CC higher *Lactobacillus* and lower *Clostridiaceae* unc, *Ruminococcus*, *Barnesiella* vs. control
Non–murine models				
Cheng, 2018 (30)	High, medium, or low dose B12 (with low-dose FA)	Diversity decreased for low to high B12 dose for OTUs, ACE, and Chao1 index; diversity was inverted U-shaped (highest for medium B12 dose) based on Simpson and Shannon index[2]	Clustering based on diet (UPGMA clustering tree, PCoA): B12-med tended to cluster away from B12-low and B12-high[2]	Phylum: B12-med had highest Firmicutes and lowest Proteobacteria Genera: B12-low had highest *Desulfovibrio*, B12-med had highest abundance of *Bacteroides*, B12-high had highest abundance of *Barnesiella*

(*Continued*)

TABLE 6 (*Continued*)

First author, year (ref)	Comparison	Alpha-diversity	Beta-diversity	Relative abundance
	High-, medium-, or low-dose B12 (with high-dose FA)	Diversity was U-shaped (lowest for medium B12 dose) based on OTU numbers, ACE index, and Chao1; diversity increased from low to high B12 dose based on Simpson and Shannon index[2]		Phylum: B12-low had the highest Proteobacteria; B12-med had highest Firmicutes and lowest Proteobacteria; B12-high had highest Bacteroidetes, Cyanobacteria, and Verrucomicrobia Genera: B12-low and B12-med had the highest *Desulfovibrio*, B12-low had highest *Bacillus*, B12-high had highest *Alistipes*
Long, 2018 (31)	Increasing doses of B12, from 0 mg/kg (group 1) to 0.025 mg/kg (group 6)	Group 3 had highest diversity and group 5 had lowest diversity based on OTUs, ACE, Chao1, Shannon, and Simpson index[2]	Group 1 and 6 clustered together, group 1 and 5 were clustered furthest[2]	Class: Bacteroidia (highest in 6; lowest in 2), Clostridium (highest in 4; lowest in 5), Gamma-proteobacteria (highest in 5; lowest in 6), Melainabacteria (highest in 3; lowest in 2), Deferribacteres (highest in 2; lowest in 1 and 6) Family: *Bacteroidaceae* (highest in 5 vs. 1 and 4; lowest in 4), *Ruminococcaceae* (highest in 3 vs. 2), *Desulfovibrionanceae* (highest in 5; lowest in 6), *Prevotellaceae* (highest in 1 vs. 2 and 4 only), *Verrucomicrobiaceae* (highest in group 3 vs. group 2) Genus: *Bacteroides* (highest in 5 vs. 1 and 4; lowest in 4), *Desulfovibrio* (highest in 5; lowest in 6), *Alistipes* (highest in 4 vs. 1 and 2), *Muscispirillum* (highest in 2)
Duan, 2021 (32)	Methylmalonic acid concentrations	Not reported	Not reported	Genus: Higher *Formosa*, *Saccharibacteria_norank*, *Kriegella*, *Pontibaca*, *Rhodobacteraceae_unclassified*, *Rhodopirellula*; and *Maribacter*, *Lutimonas*, *Ruegeria*, *Gammaproteobactereia Incertae Sedis_uncultured*, *Flavobacteriaceae_unclassified*, *Muricauda*; and lower *Weissella*

[1] ACE, abundance-based coverage estimator; B12, vitamin B-12; CC, cyanocobalamin; MC, methylcobalamin; med, medium; OTU, operational taxonomic unit; PCoA, principal coordinates analysis; ref, reference; unc, unclassified; UPGMA, unweighted pair group method with arithmetic mean.

[2] No statistical test reported, based on study authors or systematic review authors interpretation of figures.

did not change in the other groups (0 and 200 mg/kg). Within this family, higher serum vitamin B-12 concentrations were associated with lower relative abundance of *Clostridium* cluster XIVb and higher *Clostridium* cluster XIVa in rats (29).

Proteobacteria phylum. Within the family *Desulfovibrionaceae* (Deltaproteobacteria class), the relative abundance of *Bilophila* increased in mice receiving 0 and 50 mg/kg, but not 200 mg/kg, of cyanocobalamin (27), and *Desulfovibrio* was highest among geese receiving the lower vitamin B-12 dose (0.009 mg/kg) (30). The relative abundance of the class Alphaproteobacteria was higher in vitamin B-12–deficient rats compared with the control group (28); within this class, higher MMA concentrations (i.e., lower vitamin B-12 status) in shrimp were associated with increased abundance of several genera in the *Rhodobacteraceae* family (32).

Bacterial function.

Two of the included studies reported data on functional outcomes (26, 29). In 1 study (26), there were no significant differences in predicted genes between intervention groups (i.e., control, cyanocobalamin, methylcobalamin), including genes related to vitamin B-12. In another study (29), higher vitamin B-12 concentrations in rats were associated with lower capacity for energy and carbohydrate metabolism pathways.

Other outcomes.

In 1 study in mice (25), there were no significant effects of cyanocobalamin on acetate, propionate, or butyrate concentrations in cecum; cobinamide and cobalamin concentrations in stool increased in the group that received cyanocobalamin but not the control group. Two studies in mice found no differences in the colonic expression of inflammatory cytokines between cyanocobalamin supplementation and control groups (25, 27). In proteomics analyses in a study in rats (29), higher vitamin B-12 concentrations were associated with concentrations of several serum proteins (Table 5).

Qualitative synthesis of evidence from observational human studies

Findings from the 12 observational studies in humans are summarized in **Table 7** (primary outcomes) and in Table 5 (secondary outcomes).

Alpha-diversity.

Four of the observational human studies reported results for alpha-diversity (35, 36, 40, 43). Vitamin B-12 intake was associated with increased alpha-diversity in adults; however, vitamin B-12 intake or status was not associated with alpha-diversity in infants or children. In a cohort study in lactating women (40), the Simpson evenness index was significantly higher among lactating females in the third quartile of vitamin B-12 intake (3.0–6.3 μg/d), compared with the other quartiles. In a study in older male veterans (50–75 y) (35), higher vitamin B-12 intake was associated with a higher Shannon index. In contrast, alpha-diversity (Shannon's diversity index, observed OTUs) did not differ between vitamin B-12–sufficient ($\geq$150 pmol/L) and –deficient ($<$150 pmol/L) infants, or among vitamin B-12–deficient infants before and after intramuscular vitamin B-12 injection (43). A study among children (36) reported that vitamin B-12 intake was not associated with Shannon index, phylogenetic diversity, or richness.

Beta-diversity.

Four observational studies reported heterogenous findings for beta-diversity (35, 36, 40, 43). There were no differences in beta-diversity of the gut microbiota between vitamin B-12–deficient and –sufficient infants, or in vitamin B-12–deficient infants before and after intramuscular vitamin B-12 injection (43). However, when stratified by age, beta-diversity differed by vitamin B-12 deficiency in infants at 6 mo of age, but not at 4 or 5 mo of age. The beta-diversity of the gut microbiota did not differ by vitamin B-12 intake in children (36) or by quartiles of vitamin B-12 intake in a study in lactating women (40). However, beta-diversity differed by median vitamin B-12 intake in a study in older male veterans (35).

Bacterial abundance.

Four studies in adults reported associations between vitamin B-12 intake and bacterial abundance at the phylum level (33, 35, 40, 42). Higher vitamin B-12 intake was associated with lower relative abundance of Bacteroidetes (42) and higher relative abundance of Proteobacteria (40) and Verrucomicrobia (35). One study in women (33) reported a differential abundance of Proteobacteria by median vitamin B-12 intake, but the direction of this association was not reported.

Bacteroidetes phylum. Vitamin B-12 intake was associated with a lower relative abundance of *Bacteroides* in studies in older male veterans (35) and lactating women (40). Additionally, *Odoribacteraceae* was differentially abundant among women by median vitamin B-12 intake (33), and higher vitamin B-12 intake was associated with higher relative abundance of the genus *Odoribacter* in older male veterans (35). In a study in lactating women (40), vitamin B-12 intake was associated with a higher abundance of *Prevotella*; in contrast, in a study in breastfed infants, higher vitamin B-12 status was associated with a lower abundance of this genus (43). Vitamin B-12 intake in older male veterans was also associated with a greater abundance of *Alistipes* (35).

Firmicutes phylum. The relative abundance of *Ruminococcaceae* was differentially enriched in women with vitamin B-12 intake below the median (33). Higher vitamin B-12 intake in adults (35, 40) and higher vitamin B-12 status in breastfed infants (43) were associated with a greater abundance of *Faecalibacterium* (*Ruminococcaceae* family), and maternal vitamin B-12

TABLE 7 Human studies assessing the association between vitamin B-12 and the gut microbiome[1]

First author, year (ref)	Comparison	Alpha-diversity	Beta-diversity	Relative abundance
Boran, 2020 (43)	B12 sufficient vs. deficient infants	Not significant (no differences when stratified by age, sex, history of probiotic use, or delivery mode)	Significant clustering when stratified by age among infants at 6 mo (but not 4 or 5 mo) in unweighted UniFrac	Phylum, genus: not significant (no difference when stratified by age, sex, history of probiotic use, delivery mode)
	B12 concentrations	Not reported	Not reported	Phylum: not significant Genus: higher *Blautia*, *Faecalibacterium*, *Fusicatenibacter*, *Lachnospira*, and *Lachnospiraceae (FNWNL329)*; lower *Bilophilia*, *Clostridiales (UncAna95)*, *Lawsonella*, and *Prevotella*
	Post- vs. pre- B12 intravenous injections	Not significant	No significant clustering	Phylum, genus: not significant
Babakobi, 2020 (41)	Maternal B12 intake	Not reported	Not reported	Family, genus: not significant (infant gut microbiome)
Carrothers, 2015 (40)	B12 intake	Higher in quartile 3 (3.0–6.3 μg/d) vs. other quartiles (Simpson evenness)	No clustering by B12 quartiles	Phylum: Higher Proteobacteria among individuals consuming the highest quartile of B12 intake (6.4–18.4 μg/d) compared with the other quartiles Genus: lower *Bacteroides*; higher *Prevotella*
Gurwara, 2019 (35)	B12 intake	Higher (Shannon index; continuous B12)	Significant clustering above vs. below median B12 intake	Phylum: higher Verrucomicrobia (above vs. below median) Genus: higher *Faecalibacterium*, *Roseburia*, *Alistipes*, *Odoribacterium*, *Dialister*, *Akkermansia*, *Haemophilus*; and lower *Erysipelatoclostridium*, *Bacteroides*, *Lachnospiraceae* (*UncO8895*), *Lachnoclostridium* (above vs. below median) Genus: higher *Faecalibacterium*, *Akkermansia*, *Haemophilus*, and lower *Bacteroides* and *Lachnospiraceae* (*Unc94789*) (continuous B12) Genus: higher *Faecalibacterium*, *Dialister*, *Roseburia*; and lower *Erysipelatoclostridium* (continuous B12 adjusted for age, ethnicity, smoking status, alcohol consumption, BMI, diabetes, and hypertension)
Herman, 2020 (36)	B12 intake	Not significant	No significant clustering	Genus: not significant
Mörkl, 2018 (33)	B12 intake (above vs. below median)	Not reported	Not reported	Differentially abundant bacteria: Proteobacteria, Archea, *Odoribacteriaceae*, *Clostridia* Differentially abundant with B12 below median: *Ruminococcaceae*
Selma-Royo, 2021 (37)	Maternal B12 intake	Not reported	Not reported	Higher in infants: *Klebsiella*, *Bifidobacteria*, *Streptococcus*, *Enterococcus*, *Dorea*, *Faecalibacterium*, *Agathabacter*
Seura, 2017 (38)	B12 intake	Not reported	Not reported	Not significant
Shah, 2017 (42)	B12 intake	Not reported	Not reported	Phylum: lower Bacteroidetes
Tamura, 2017 (34)	B12 intake	Not reported	Not reported	Family: lower *Enterobacteriaceae*
Valentini, 2015 (39)	Changes in B12 concentrations	Not reported	Not reported	Increased *Bifidobacteria* concentration among participants with low-grade inflammation ($r = 0.663$, $P = 0.001$) but not significant among participants without inflammation ($r = 0.068$, $P = 0.682$)

[1] B12, vitamin B-12; ref, reference.

intake (37) was associated with a higher abundance of *Faecalibacterium* in infants. In the *Clostridiaceae* family, the genus *Clostridium* was differentially abundant by median vitamin B-12 intake (33); however, the direction of this association was not specified.

Actinobacteria phylum. Increased total serum vitamin B-12 concentrations were associated with greater increases in the genus *Bifidobacteria* (16S rRNA gene copy number/ng stool) in older European adults with low-grade inflammation but not among participants without inflammation (39). Higher maternal vitamin B-12 intake was also associated with greater relative abundance of *Bifidobacteria* in infants (37).

Proteobacteria phylum. Vitamin B-12 intake was associated with a lower abundance of *Enterobacteriaceae* in older adults (65–84 y) in Japan (34). Higher maternal intake of vitamin B-12 was associated with a higher abundance of *Klebsiella* (genus within the *Enterobacteriaceae* family) in their infants (37). Within the *Desulfovibrionaceae* family, higher vitamin B-12 status in breastfed infants was associated with a lower relative abundance of *Bilophila* (43).

Four of the included studies reported no significant associations between vitamin B-12 (intake or intervention) and bacteria abundance, including a cross-sectional study in children (36), before and after intramuscular vitamin B-12 injections (i.e., bypassing intestinal absorption) among breastfed infants with vitamin B-12 deficiency (43), maternal vitamin B-12 intake in mother–infant dyads (41), and a cross-sectional study in women (20–22 y) using the terminal restriction fragment length polymorphism (T-RFLP) method to assess the gut microbiome (38).

Other outcomes.

One study in older adults in Japan (34) found that higher vitamin B-12 intake was associated with greater quercetin (a polyphenol with anti-inflammatory properties) degradation in stool samples incubated with quercetin, indicating a greater reduction in bioactivity. Another study in women in Austria (33) found that higher vitamin B-12 intake was associated with higher concentrations of zonulin, a marker of gut permeability.

Discussion

Vitamin B-12 is synthesized and utilized by bacteria in the human gut microbiome. However, the impact of vitamin B-12 on the gut microbiome has not been fully established. To our knowledge, this is the first systematic review to date, to assess the impact of vitamin B-12 status on the gut microbiome. Nineteen of the 22 included studies reported vitamin B-12 intake, status, or supplementation was associated with gut microbiome outcomes, including alpha-diversity and beta-diversity, relative abundance of bacteria, functional capacity, or SCFA production (**Table 8**; summary of overall findings). Findings from in vitro, animal, and human studies suggest that vitamin B-12 may be associated with changes in bacterial abundance. While evidence from in vitro studies indicates that vitamin B-12 may increase alpha-diversity and shift gut microbiome composition (beta-diversity), results from animal studies and observational human studies were heterogeneous. Findings from laboratory studies suggested that the impact of vitamin B-12 supplementation on the gut microbiome may differ by cobalamin form and co-intervention. To date, few prospective studies and no randomized trials have been conducted to examine the effects of vitamin B-12 on the gut microbiome.

Gut microbiome alpha-diversity and beta-diversity

Findings regarding the associations between vitamin B-12 and alpha-diversity were heterogeneous, and varied with cobalamin form (22, 23), index used (23), and co-interventions (22–24, 30). Higher alpha-diversity reflects a redundancy in gut microbiome function and stability. Lower alpha-diversity has been associated with cardiometabolic outcomes (45–49) and undernutrition (45–47). Given the association of lower alpha-diversity with adverse health outcomes, further understanding of the potential role of vitamin B-12 in gut microbial richness and evenness is warranted.

Vitamin B-12 was associated with shifts in beta-diversity in some studies (22–24, 30, 31, 35, 43), suggesting that vitamin B-12 may modulate gut microbiome composition, although evidence was mixed (26–28, 36, 40). Beta-diversity has been used to differentiate the microbial community structure between healthy controls and individuals in diseases such as inflammatory bowel disease (48), colorectal cancer, liver cirrhosis (49), obesity (49, 50), and type 2 diabetes (49, 51). Results for beta-diversity and alpha-diversity suggest that vitamin B-12 may alter gut microbiome composition; however, host factors, form of cobalamin, and co-interventions may affect the direction and magnitude of these changes and need to be elucidated.

Phyla abundances

In studies reporting results at the phylum level, vitamin B-12 intake or interventions were associated with higher Firmicutes (26) and Proteobacteria (24, 26, 33, 40) and lower Bacteroidetes (24, 26, 42) and Actinobacteria (28)—or the opposite direction of these trends (22, 24, 30). The relative abundance of phyla, such as lower Bacteroidetes:Firmicutes ratio, have been associated with obesity (51–54) and type 2 diabetes (51); however, this association is not consistent in all populations, and reasons for the discrepancy are unclear (55). Further studies are needed to understand these shifts at the phylum level and subsequent health outcomes.

Genera abundances

Relative abundance of genera has been used to characterize the gut microbiome into enterotypes (56, 57), and long-term diet has been identified as a main driver of enterotype classification (58). Changes from short-term diet and interventions may differ by baseline enterotype.

TABLE 8 Summary of findings from included studies assessing the association between vitamin B-12 and the gut microbiome[1]

Outcome	In vitro ($n = 3$)	Animal models ($n = 8$)		Human observational ($n = 11$)
		Murine ($n = 5$)	Non-murine ($n = 3$)	
Alpha-diversity	↑ depending on co-intervention, diversity index, and cobalamin form ($n = 3$)	↓ ($n = 1$) or – ($n = 2$)	↑, ↓, or U-shaped depending on co-intervention and diversity index ($n = 2$)	↑ Diversity ($n = 2$, in adults); – ($n = 2$, in children)
Beta-diversity	Yes, depending on co-intervention ($n = 3$)	Yes ($n = 1$) or no ($n = 3$)	Yes depending on co-intervention ($n = 1$) or U-shaped ($n = 1$)	Yes ($n = 2$, diet in men, status in infants) or no ($n = 2$, diet in women and children)
Phyla	Heterogenous results for Bacteroidetes ($n = 1$), Firmicutes ($n = 1$), and Proteobacteria ($n = 2$) depending on co-intervention; ↑ Verrucomicrobia ($n = 1$)	↓ Firmicutes and Proteobacteria (with CC), ↑ Firmicutes and Proteobacteria (with MC) ($n = 1$)	↑ Bacteroidetes, Firmicutes, Verrucomicrobia, and ↓ Proteobacteria depending on cointervention ($n = 1$)	↓ Bacteroidetes ($n = 1$), ↑ Proteobacteria ($n = 1$), and ↑ Verrucomicrobia ($n = 1$)
Genera	↓ *Bacteroides* ($n = 2$), *Bifidobacteria* ($n = 1$) ↑ *Klebsiella* ($n = 1$), *Acinetobacter* ($n = 2$)	↓ *Bacteroides* ($n = 2$)	Heterogenous results for *Bacteroides*, depending on co-intervention ($n = 1$)	↓ *Bacteroides* ($n = 2$) ↑ *Bifidobacteria* ($n = 2$), *Faecalibacterium* ($n = 3$), *Klebsiella* ($n = 1$)
Functional outcomes	↑ Lipid metabolism and degradation of exogenous substances ($n = 2$), ↓ carbohydrate metabolism pathways ($n = 1$); ↑ SCFAs depending on cointervention and cobalamin form ($n = 2$)	↓ Carbohydrate metabolism pathways ($n = 1$); – SCFAs ($n = 1$)	—	↑ Serum zonulin ($n = 1$) and ↓ quercetin degradation ($n = 1$)
Summary of research gaps	• Several methodological differences with study types (e.g., cobalamin form, dose, and co-interventions) • Few studies ($n = 1$ in murine) used whole-genome shotgun sequencing • No studies in humans used whole-genome shotgun sequencing or assessed functional capacity • No randomized controlled trials have been conducted in humans, and few prospective studies ($n = 5$) with limited follow-up (<3 months) and data reported at 1 or 2 time points • Few studies in humans assessed vitamin B-12 status ($n = 2$), and used only 1 biomarker • No studies have been conducted in human populations with high prevalence of vitamin B-12 deficiency			

[1] CC, cyanocobalamin; MC, methylcobalamin; *n*, number of studies; ↑, increased; ↓, decreased; –, no difference.

Bacteroides.
In several studies, higher vitamin B-12 intakes in humans (35, 40) or vitamin B-12 supplementation in rodents (25, 27) and in vitro models (23, 24) were associated with a lower relative abundance of *Bacteroides*. *Bacteroides* encodes several vitamin B-12 transporters (18), suggesting that they may have an advantage in utilizing vitamin B-12 in the gut environment. However, a vitamin B-12–rich environment may hinder this competitive advantage (25). *Bacteroides*, a Gram-negative genus of the Bacteroidetes phylum, can metabolize glycans and polysaccharides in the gut and have both commensal and pathogenic implications for immune function (59) and metabolic disease (52, 60).

Faecalibacterium.
Higher vitamin B-12 intake (35, 37) and status (43) were associated with increased relative abundance of *Faecalibacterium*. Although 1 study found no association with vitamin B-12 intake (40), the 24-h recall used may not reflect habitual intake. No studies in murine or in vitro models reported associations with *Faecalibacterium*, which may be due to a lower abundance of *Faecalibacterium* in rodents compared with humans (61). A recent metabolic reconstruction of *F. prausnitzii*'s metabolism found that it is not able to produce cobalamin (62), suggesting increased growth in a vitamin B-12–replete environment. *Faecalibacterium is* a Gram-positive genus in the Firmicutes phylum that includes *F. prausnitzii*, a commensal taxon that is one of the most abundant microbial species in humans and an important contributor to gut microbiome function and production of butyrate, an SCFA with anti-inflammatory effects (63).

Differences by cobalamin form

Findings from in vitro and animal studies suggest that specific cobalamin forms may differentially impact alpha-diversity and beta-diversity and bacterial abundance. Although in humans, all cobalamin forms are interconverted and transported within the cell (64), different forms of cobalamin may have distinct roles in bacteria metabolism. Differences in cellular transport among forms is unlikely, as the predominant transporter, an ATP-binding cassette (ABC)-type BtuFCD transport system, recognizes the lower ligand of cobalamin that is shared among all forms (18). In contrast, cobalamin forms may differ in enzyme affinity (i.e., adenosylcobalamin-dependent enzymes) or ability to control gene expression through riboswitches—regulatory elements of mRNA.

Most cobalamin riboswitches are thought to be adenosylcobalamin-dependent, as demonstrated in some model bacterial species (65–67), but some *Escherichia coli* riboswitches have over 500 times higher affinity for methylcobalamin and aquocobalamin, compared with adenosylcobalamin (68). Zhu et al. (26) found that cyanocobalamin improved growth and increased Shiga toxin production of *E. coli* compared with methylcobalamin, whereas *Lactobacillus reuti* had similar growth with both cobalamin forms. Cyanocobalamin and methylcobalamin also had different effects on enzyme activity, and cyanocobalamin had greater inhibition of riboswitch expression. In contrast, in the *Propionibacterium* strain UF1, cyanocobalamin, methylcobalamin, hydroxycobalamin, and adenosylcobalamin had similar regulation of genes involved in vitamin B-12 biosynthesis (69). As genomic studies elucidate mechanisms of gene regulation in bacteria, *in vivo* studies are needed to determine how different cobalamin forms impact the bacterial community.

Limitations

This systematic review has several limitations. The lack of randomized trials and few prospective studies conducted in humans constrain the interpretability of findings and causal inference. Methodological differences among the included studies, such as sequencing techniques (e.g., targeted, 16S rRNA gene, metagenome) and microbiome samples (e.g., stool in humans, cecum contents in animals), and heterogeneity in model organisms (i.e., rodents, birds, shrimp), population characteristics (e.g., age, sex), and vitamin B-12 interventions and exposures limit interpretation and comparability of findings. Studies have demonstrated that gut microbiome varies by age (70–72) and host organism (61, 73, 74), while differences between methods can explain a larger variation than biological differences (75, 76). Detection of subtle effects on the gut microbiome requires use of consistent methods (77, 78).

Research gaps and future directions

Study design.
Randomized trials are needed to determine the effects of vitamin B-12 supplementation on the human gut microbiome, particularly in populations with variation in baseline vitamin B-12 status. Large randomized trials and controlled-feeding studies that assess the effects of low-dose supplements or fortification, would further inform public health interventions. Prospective studies with both dietary intake and biomarkers of vitamin B-12 status, and consideration of confounding variables, would enhance inference and comparability between studies.

Cross-population replication.
Studies in a specific population (i.e., age, sex, country) need to be replicated in different microbiome backgrounds to determine which associations between vitamin B-12 and the gut microbiome are consistent, as previously demonstrated for microbiota–disease associations in varying geographic locations in China (79).

Vitamin B-12 and microbiome assessment.
Dietary analyses that evaluate food groups and other individual nutrients typically consumed with vitamin B-12 could help to differentiate observed associations from other dietary components. In addition to total vitamin B-12, analysis of other circulating (i.e., holotranscobalamin) and functional (i.e., MMA) biomarkers would improve assessment of vitamin B-12 status. Leveraging advances in the field of

microbiome research (e.g., -omics data, high-dimensional statistics) would allow for investigation of functional changes in the gut microbiome and implications for human health.

Additional nutrients in one-carbon metabolism.
B-vitamins and other nutrients involved in one-carbon metabolism can also be synthesized and utilized by gut bacteria (14, 80–83). Folate status, intake, and/or supplementation (28, 30, 39, 84–90) may influence relative bacterial abundance and other gut microbiome outcomes. Other B-vitamins, such as riboflavin (40, 85, 91–93), niacin (94), vitamin B-5 (40), and vitamin B-6 (40, 85, 95), and choline (85, 96, 97) and methionine (85, 98, 99) have also been associated with changes in relative bacterial abundance.

Potential mechanisms. Modulation of the gut microbiome by vitamin B-12 or other nutrients in one-carbon metabolism may occur via several potential mechanisms. The impact of vitamin B-12 on the gut microbiome may be explained by vitamin B-12–dependent enzymes and riboswitches (14, 18); however, the mechanisms that link vitamin B-12 and the human gut microbiome have not been fully established. *One-carbon metabolism.* The role of other nutrients in one-carbon metabolism may provide further insights into the links between vitamin B-12 and the gut microbiome. For example, SAM production (i.e., a methyl donor and product of one-carbon metabolism) can be used for DNA methylation in bacteria to alter gene expression (100) or in mucosal cells, which could impact the intestinal environment (99, 101, 102). SAM is also a substrate for production of metabolites used in communication between bacteria (i.e., quorum sensing) (103, 104). Through B-vitamin sharing among bacteria in the gut microbiome (20), bacteria may benefit from an abundance or stability of B-vitamin availability in the gut environment (105). Additionally, products of B-vitamin metabolism may be used by other bacteria in the gut (20, 105, 106), which may offer competitive advantage to some bacteria without B-vitamin–dependent enzymes. *Other mechanisms.* Choline can be metabolized by bacteria in the gut microbiome to produce trimethylamine (82), which may decrease choline availability for the host (107) and limit production of SAM. Additionally, niacin may have a role in decreasing bacterial endotoxin production and improving intestinal barrier function (108, 109), and Steinert and colleagues (92, 110) hypothesized that riboflavin may reduce oxidative stress via NADH-redox reactions in bacteria.

Alterations in one-carbon metabolism through deficiency in 1 or more nutrients could interact with vitamin B-12 to impact gut microbiome composition and function. Future research is needed to examine the independent effects of vitamin B-12 and potential interactions with folate and other nutrients in shaping the gut microbiome.

Translational research.
In vitro and animal models differ from the human gastrointestinal tract—in terms of structure, transit time, abundance of some bacteria, and sample type (61, 73, 74). However, laboratory-based models provide a controlled environment to evaluate the effects of vitamin B-12 on the gut microbiome, which can inform mechanistic hypotheses and studies in humans. Given the findings from experimental in vitro and animal models and observational human studies on vitamin B-12 and gut microbiome, further evidence is needed from mechanistic studies and randomized trials in human populations.

Impact of microbiome on vitamin B-12 status.
The studies included in this review focused on the impact of vitamin B-12 on modulating the gut microbiome. However, few studies have evaluated the potential impact of gut bacteria on host vitamin B-12 status (14, 80, 111, 112). One study estimated that 42% of the human gut microbiome genome synthesizes vitamin B-12, and the human gut microbiome has the capacity to produce approximately one-third of the daily recommended intake of vitamin B-12 (80). However, only approximately 2% of corrinoids found in human feces are cobalamin, while the remainder are vitamin B-12 analogs (16), suggesting that vitamin B-12 analogs produced by bacteria are more likely to enter the circulation than vitamin B-12. Another study found that elevated MMA (>0.75 mmol/L) was associated with higher concentrations of vitamin B-12 analogs bound to holohaptocorrin, the circulating form of vitamin B-12 thought to be inactive (112), suggesting that concentrations of vitamin B-12 analogs could impact vitamin B-12 status.

In humans, vitamin B-12 transporters are found only in the small intestine; vitamin B-12 analogs produced by bacteria in the large intestine would likely enter the circulation via passive diffusion (14). However, passive diffusion of vitamin B-12 through the small intestine only accounts for 1–2% of an oral dose (15), so passive diffusion in the large intestines may not account for a substantial amount of absorption. Stable-isotope methods could elucidate the potential impact of the gut microbiome on host vitamin B-12 status.

Clinical and public health implications.
Further understanding of the impact of vitamin B-12 on the gut microbiome is needed to inform clinical and public health interventions. Vitamin B-12 supplementation may be beneficial to gastrointestinal conditions, beyond treating malabsorption-induced vitamin B-12 deficiency, through interactions with the gut microbiome. In colitis-induced mice, methylcobalamin supplementation (25, 26), but not cyanocobalamin (26), yielded improvements in disease activity score and colonic weight loss (26). This could be due to gut microbial production of propionate, which has a demonstrated role in gut integrity and host health (63): in gut simulators, methylcobalamin supplementation, but not cyanocobalamin, increased propionate production (23). Although intramuscular vitamin B-12 is most commonly used to treat vitamin B-12 deficiency in individuals with malabsorption disorders, some studies have found that oral doses of vitamin B-12 are as effective (113), and may incur

additional benefits via the gut microbiome. Understanding the mechanisms and impact of vitamin B-12 on gastrointestinal health could help to inform recommendations for co-interventions.

Conclusions

Vitamin B-12 is synthesized and utilized by bacteria in the human gut microbiome. However, the impact of vitamin B-12 on the gut microbiome has not been established. Evidence from laboratory studies suggests that vitamin B-12 may be associated with changes in bacterial abundance and diversity, but may differ by cobalamin form, co-interventions, or other host factors. However, overall findings from observational human studies and in vitro and animal studies regarding alpha-diversity and beta-diversity, bacterial abundances, and function are heterogeneous. To date, few prospective studies and no randomized trials have been conducted to determine the effects of vitamin B-12 on the human gut microbiome. The impact of vitamin B-12 on the gut microbiome needs to be elucidated to inform public health and clinical interventions.

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Conflicts of interest

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