Vitamin B_{12} sources and microbial interaction

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Impact statement

To prevent vitamin B_{12} (B_{12}) deficiency in high-risk populations such as vegetarians and elderly subjects, it is necessary to identify foods that contain high levels of B_{12}. B_{12} is synthesized by only certain bacteria and archaeon, but not by plants or animals. The synthesized B_{12} is transferred and accumulated in animal tissues, even in certain plant tissues *via* microbial interaction. Meats and milks of herbivorous ruminant animals are good sources of B_{12} for humans. Ruminants acquire the essential B_{12} through a symbiotic relationship with bacteria inside the body. Thus, we also depend on B_{12}-producing bacteria located in ruminant stomachs. While edible plants and mushrooms rarely contain a considerable amount of B_{12}, mainly due to concomitant bacteria in soil and/or their aerial surfaces. In this mini-review, we described up-to-date information on B_{12} sources and bioavailability with reference to the interaction of microbes as B_{12}-producers.

Abstract

Vitamin B_{12} is synthesized only by certain bacteria and archaeon, but not by plants. The synthesized vitamin B_{12} is transferred and accumulates in animal tissues, which can occur in certain plant and mushroom species through microbial interaction. In particular, the meat and milk of herbivorous ruminant animals (e.g. cattle and sheep) are good sources of vitamin B_{12} for humans. Ruminants acquire vitamin B_{12}, which is considered an essential nutrient, through a symbiotic relationship with the bacteria present in their stomachs. In aquatic environments, most phytoplankton acquire vitamin B_{12} through a symbiotic relationship with bacteria, and they become food for larval fish and bivalves. Edible plants and mushrooms rarely contain a considerable amount of vitamin B_{12}, mainly due to concomitant bacteria in soil and/or their aerial surfaces. Thus, humans acquire vitamin B_{12} formed by microbial interaction *via* mainly ruminants and fish (or shellfish) as food sources. In this review, up-to-date information on vitamin B_{12} sources and bioavailability are also discussed.

Keywords: Bioavailability, cobalamin, food source, microbial interaction, ruminant animals, vitamin B_{12}

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Introduction

Vitamin B_{12} (B_{12}) or cyanocobalamin is a member of the corrinoids that contain a corrin ring (Figure 1). Hydroxocobalamin, methylcobalamin, and 5′-deoxyadenosylcobalamin are chemically more labile than cyanocobalamin.[1] In particular, methylcobalamin is the cofactor of methionine synthase (EC 2.1.1.13), and 5′-deoxyadenosylcobalamin functions as the coenzyme of methylmalonyl-CoA mutase (EC 5.4.99.2), which catalyzes the conversion of (*R*)-methylmalonyl-CoA to succinyl-CoA in the catabolic pathway of amino acids and odd-chain fatty acids in mammals.[2,3]

B_{12} is synthesized by certain bacteria and archaeon, but not by plants or animals.[4] Thus, B_{12}-synthesing bacteria (including archaeon) are sources of B_{12} compounds found in foods. Both aerobic[5] and anaerobic[6] biosynthetic pathways of B_{12} compounds exist. The lower ligand is attached to the cobalt-coordinated corrin ring *via* the nucleotide loop, and 5,6-dimethylbenzimidazole is usually found as a base. Anaerobic microorganisms can synthesize corrinoids carrying bases other than 5,6-dimethylbenzimidazole.[7] Other than B_{12}, pseudovitamin B_{12} (pseudoB_{12}), which contains adenine as a base, is the only cobamide found commonly in food.[8] 5-Methoxybenzimidazolyl and 2-methylmercaptoadenyl cobamides are found in escargots.[9]

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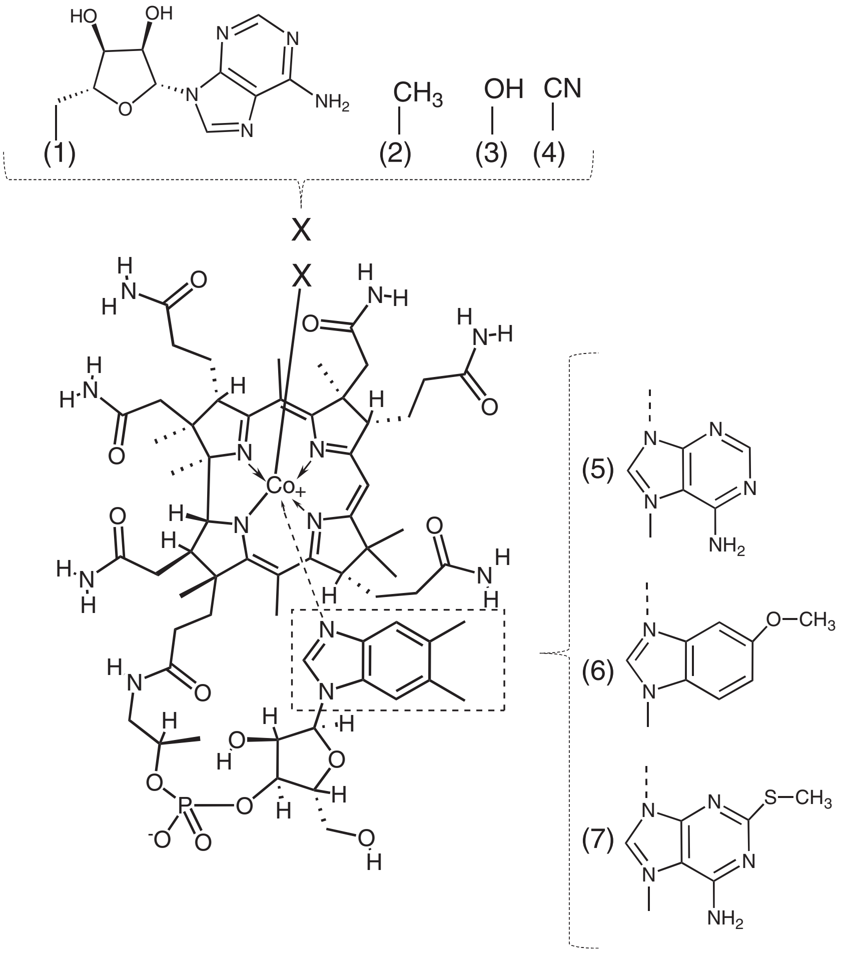

Figure 1. Structural formula of vitamin B_{12} and partial structures of vitamin B_{12}-related compounds. (1) 5′-Deoxyadenosylcobalamin, (2) methylcobalamin (3) hydroxocobalamin, (4) cyanocobalamin (vitamin B_{12}), (5) pseudovitamin B_{12}, (6) 5-methoxybenzimidazolyl cobamide, and (7) 2-methylmercaptoadenyl cobamide.

Ten years has passed since publication of my initial review concerning B_{12} sources and bioavailability[10] in this journal. For the last 10 years, liquid chromatography/electrospray ionization–tandem mass spectrometry has been widely used to analyze B_{12} compounds, and various corrinoid compounds have been newly identified from food.[8] In this mini-review, we describe up-to-date information on B_{12} sources and bioavailability with reference to the interaction of microbes as B_{12} producers.

Vitamin B_{12} in animal-derived foods

Many studies concerning the association between dietary B_{12} sources and serum (or plasma) B_{12} levels (as a marker of B_{12} status) indicate that meat, milk, and fish are associated with higher serum (or plasma) B_{12}, particularly in western countries.[11] Indeed, milk has been reported as the most important source of B_{12} for increasing serum B_{12} levels.[11–13] Various types of animal meats (e.g. beef, veal, mutton, and lamb) are derived from the muscles of ruminant animals (e.g. cattle and sheep). The remaining major meats (pork and poultry) are derived from omnivorous animals (pig and chicken) (Figure 2). Bovine milk and fermented milk (e.g. yogurt and cheese) are widely available dairy products and good B_{12} sources.[11]

Cattle and sheep are herbivores and eat plants like grass, which is free of B_{12}. These ruminants have stomachs consisting of four chambers that contain various microorganisms, including B_{12}-synthesizing bacteria.[14,15] The B_{12} synthesized in the stomach is absorbed in the intestine, transferred into the blood and stored in the liver and muscles of the animal or secreted into the milk. The cobalt content of the diet is the most important factor affecting the synthesis of B_{12} in ruminant microorganisms.[16] Thus, cobalt-deficiency readily induces B_{12}-deficiency in ruminants.[17] To enrich the B_{12} content of meat and milk, various methods for increasing ruminant B_{12} synthesis have been investigated.[18–20] Pigs and chickens are omnivores and eat both plants and animals, which are B_{12} sources. The B_{12} content of raw meat is generally higher in these ruminants than in pig or chicken,[21] although the B_{12} content of poultry meat may be increased by the administration of lactic bacteria.[22] Chicken egg consumption does not appear to significantly contribute to higher serum B_{12} in humans.[11]

Meat

Raw livers of beef, pork, and chicken contain high B_{12} (52.8, 25.2, and 44.4 μg/100 g wet weight, respectively)[21] and are excellent sources of B_{12}. The B_{12} content of raw meats

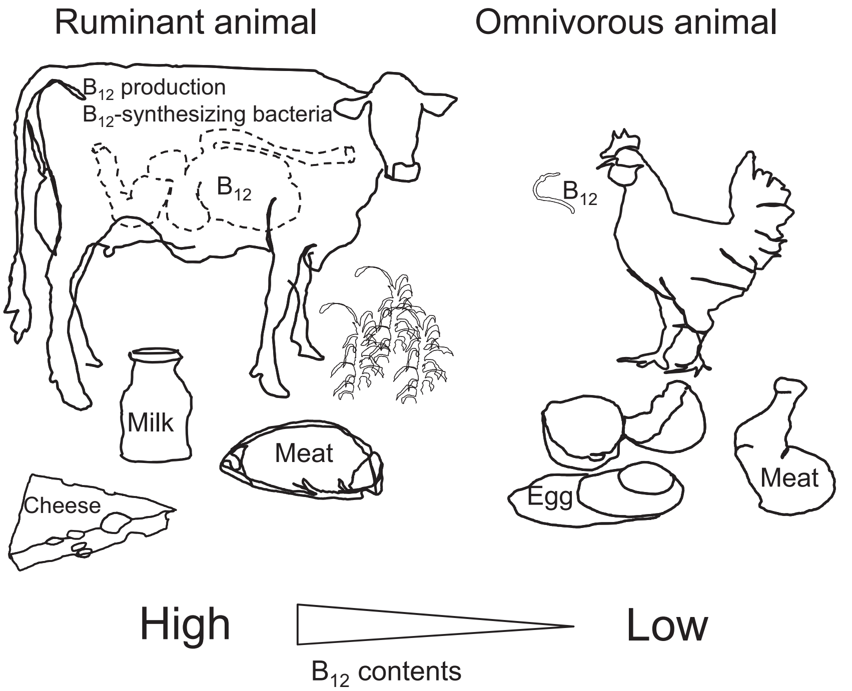

Figure 2. Vitamin B_{12} sources and microbial interactions in meat, eggs, milk, and milk products. Cattle are herbivorous ruminant animals and their stomachs contain various microorganisms including B_{12}-synthesizing bacteria. The synthesized B_{12} is absorbed in the intestine and stored in the liver and muscles of cattle or secreted into milk. Bovine milk and fermented milk (yogurt and cheese) are major dairy products for humans. Chickens are omnivores and eat both plants and animals that contain considerable amounts of B_{12}. The B_{12} contents of raw meats are generally higher in cattle than in chicken.

(approximately 1.0–2.0 μg/100 g wet weight) is higher in beef than in pork (approximately 0.5 μg/100 g wet weight) or chicken (<0.5 μg/100 g wet weight),[21,23] suggesting that the meats and livers of ruminant animals contain higher amounts of B_{12} relative to those of omnivorous animals. A considerable loss of B_{12} has been reported after cooking beef, pork, and chicken meats.[23–25] The retention of B_{12} in vacuum-cooked meats has been reported to be 100% for veal, lamb, and pork, and 87% for beef.[26] For more detailed information on animal sources of B_{12}, such as meat and dairy products, please refer to an excellent review cited in Gille and Schmid.[23]

Milk

The B_{12} concentrations in milk from ruminants such as sheep (0.71 μg/100 g of milk), cow (0.35 μg/100 g of milk), and goat (0.06 μg/100 g of milk) are higher than those found in human milk (0.04 μg/100 g of milk).[27] While the B_{12} content of bovine milk is not high relative to beef meats, bovine milk and fermented milk (e.g. yogurt and cheese) are major B_{12} sources because the intake of milk or dairy products is high in various populations.[28] The B_{12} concentration of bovine milk varies according to many factors such as the cow type, breeding state, and milking time.[29,30] B_{12} concentrations in milk from Holstein cows appears to be generally higher than those in milk from Jersey cows.[29,30] Rutten *et al.*[31] found that a single nucleotide polymorphism (SNP) along the cow genome affects the B_{12} concentration in milk (Figure 3). Although a significant association was found between 68 SNP and B_{12} content in the raw milk of 487 first-lactation cows, this SNP was not found in the genes known to be involved in B_{12} uptake or transport, implying that there are associations related to genes involved in unknown processes such as the ruminant production of B_{12} or the secretion of B_{12} by the mammary gland.[31]

The B_{12} found in bovine milk mainly binds to transcobalamin, one of the mammalian B_{12}-binding proteins located in blood,[32] whereas haptocorrin is the predominant B_{12}-binding protein in human milk.[33] The bioavailability of B_{12} in cow's milk appears to be higher than that of cyanocobalamin.[34]

When the B_{12} contents of 26 types of commercially available natural cheeses were determined,[35] the B_{12} content was higher in hard and semi-hard cheeses (approximately 2.8 μg/100 g dry basis) and washed rind cheeses (approximately 4.2 μg/100 g dry basis) than in fresh (approximately 1.2 μg/100 g dry basis) or soft (approximately 1.8 μg/100 g dry basis) cheeses. Liquid chromatography/electrospray ionization—tandem mass spectrometry analysis has indicated that B_{12} is the predominant corrinoid compound in the tested natural cheeses, but traces of unidentified corrinoid compounds were found in some of the tested cheeses.[35]

An appreciable loss of B_{12} occurs during the storage, thermal processing, and fermentation of milk.[23,25] Recently, Johns *et al.*[36] found that the rate of B_{12} loss was three times greater in chocolate-flavored milk (approximately 33.5%) than in unflavored milk (approximately 15%) during heat treatment (1 h at 100°C). The increased loss of B_{12} in chocolate-flavored milk was attributable to cocoa polyphenols that readily form peroxides.[36]

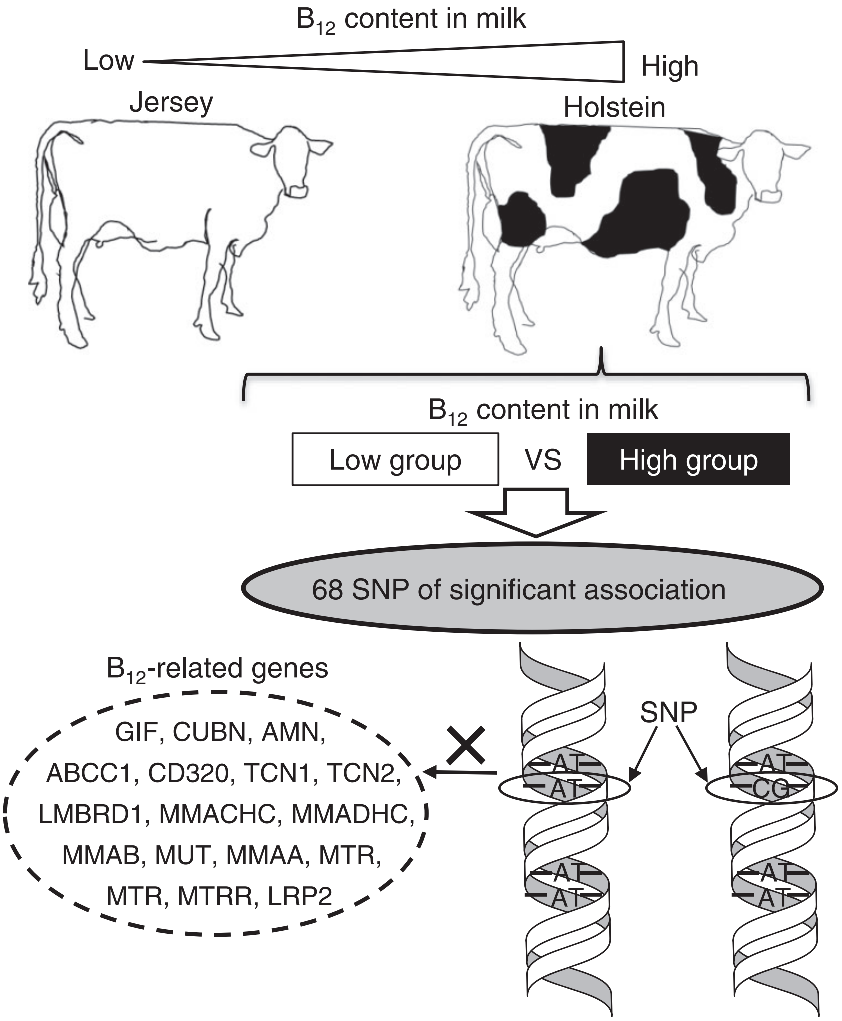

Figure 3. Single nucleotide polymorphism (SNP) and the vitamin B_{12} content of bovine milk. A significant association was found between 68 SNPs and the B_{12} content of raw milk in 487 first-lactation cows, but these SNPs were not in the following genes involved in B_{12} uptake and transport systems.
GIF: gastric intrinsic factor; CUBN: cubilin; AMN: amnionless; ABCC1: ATP-binding cassette; sub-family C; member 1; CD320: transcobalamin receptor; TCN1: transcobalaminI (haptocorin); TCN2: transcobalaminII; LMBRD1: methylmalonic aciduria cblF type 1; MMACHC: methylmalonic aciduria cblC type; MMADHC: methylmalonic aciduria cblD type; MMAB: methylmalonic aciduria cblB type; MUT: methylmalonyl CoA mutase; MMAA: methylmalonic aciduria cblA type; MTR: 5-methyltetrahydrofolate-homocysteine methyltransferase; MTRR: 5-methyltetrahydrofolate-homocysteine reductase; LRP2: low-density lipoprotein receptor-related protein 2 (megalin).

The photodegradation of vitamin B_2 is well known to occur in milk during light exposure.[37] On exposure to light, vitamin B_2 forms free radicals, which cause the color change in milk.[38] A light exposure experiment of B_{12} indicated that B_{12} is decomposed by singlet oxygen formed in an aqueous solution.[39] In addition, a B_{12} loss of 1–27% in commercially available milk products is caused by exposure to fluorescent light for 24 h at 4°C.[40] These observations suggest that storage in light accelerates the degradation of both vitamin B_2 and B_{12} in milk.

Egg

Raw and boiled whole chicken eggs contain 0.9 μg of B_{12} per 100 g wet weight of the edible portion,[21] and most of the B_{12} is located in the egg yolk.[41] Although hens have been fed B_{12}-supplemented diets to enrich B_{12} in eggs, egg yolk B_{12} levels were reportedly not changed.[42] Thus, the bioavailability of B_{12} in egg dishes is considered very low (~10%) due to the poor absorption of B_{12} of eggs.[43,44] Accordingly, egg intake does not significantly contribute to higher serum B_{12} in humans.[11]

An egg product called a century egg ("Pidan" in Chinese) is an alkaline-fermented ethnic food in China. The egg yolks of these eggs contain 1.9 ± 0.6 μg of B_{12} per 100 g wet weight. The B_{12} present in the yolk of century eggs was recovered completely in macromolecular fractions.[45] However, approximately 52% of the free B_{12} was formed from the century egg yolk during *in vitro* gastric digestion,[45] suggesting that century eggs may be a good source of B_{12}.

Fish and shellfish

People from Japan and France obtain most (84% and 64%, respectively) of their daily B_{12} intake from fish and shellfish.[46,47] Scheers *et al*.[48] indicated that serum B_{12} levels were significantly increased in subjects ingesting fish diets

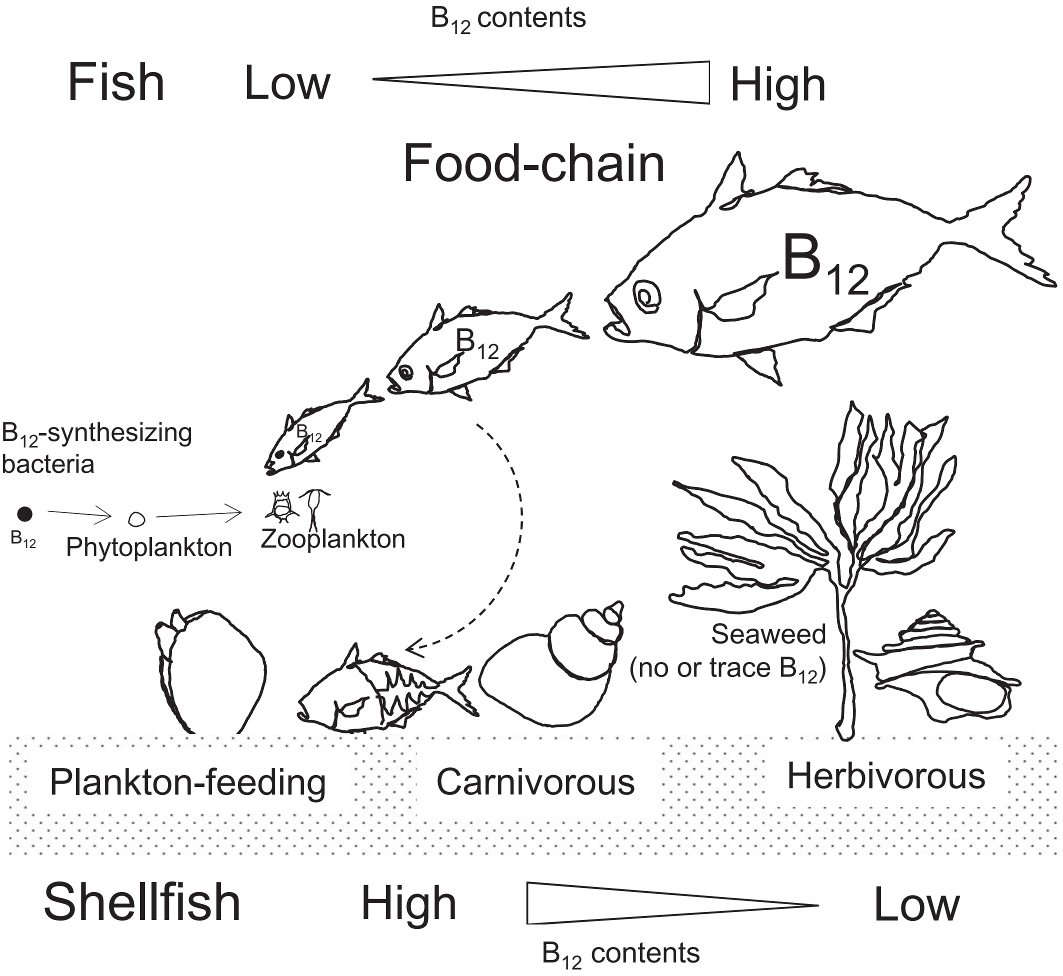

Figure 4. Vitamin B_{12} sources and microbial interactions in fish and shellfish. In aquatic environments, the B_{12} produced by certain bacteria and archaea is taken up by B_{12}-requireing bacteria as well as eukaryotic phytoplankton, most of which fall as easy prey to zooplankton. The bacterial B_{12} is transferred to fish bodies *via* plankton and concentrated in the bodies of bigger predatory fishes in the ocean food chain. B_{12} levels were significantly higher in edible bivalves that can siphon phytoplankton than in edible snails, most of which are herbivorous, eating seaweed.

compared to meat diets, suggesting that B_{12} is suitable as a marker for fish intake. Several studies have also indicated that fish and shellfish are important contributors to human B_{12} status.[11,13,48]

In aquatic environments, B_{12} produced by certain bacteria and archaea is taken up by B_{12}-requiring bacteria, as well as eukaryotic phytoplankton,[49] most of which fall as easy prey to zooplankton. Metagenomic analysis has suggested that *Thaumarchaeota* is the major B_{12} producer in aquatic environments.[50] Indeed, relative to unsupplemented phytoplankton, B_{12}-supplemented phytoplankton can significantly stimulate the growth of rotifers as a food of larval fish.[51] Thus, the bacterial B_{12} is transferred to fish bodies *via* plankton and concentrated in the bodies of bigger predatory fishes in the ocean food chain (Figure 4). Thus, meat B_{12} content is generally higher in bigger carnivorous fish than in small body fish[21]: in particular, substantial amounts of B_{12} have been shown to be accumulated in the liver or kidney of tuna[52] and salmon.[53]

The amounts of B_{12} are three times greater in the viscera (approximately 37.5 μg/100 g wet weight) than in the meat (approximately 12.2 μg/100 g wet weight) of round herring.[54] Approximately 73% of total B_{12} found in the whole fish body (except for head and bones) were recovered in the meats (approximately 5.1 μg of B_{12} per one body).[54] Serum B_{12} levels of subjects consuming herring diets are significantly increased compared to meat (poultry and pork) diets[48]: because poultry and pork meats (less than 1.0 μg/100 g wet weight) are not high in B_{12}.

The B_{12} contents of round herring and skipjack tuna meats decrease up to approximately 62% and 85% by various conventional cooking.[25,48,55] However, the retention of B_{12} in vacuum-cooked fish has been reported to be 92% for salmon and 72% for cod.[26]

Shellfish, such as edible bivalves (e.g. clams, oysters, and mussels) are well known to contain substantial amounts of B_{12}.[56,57] B_{12} compounds have been purified from these bivalves and identified as B_{12}.[58–61] However, trace pseudoB_{12} and/or unidentified corrinoid compounds are rarely detected in edible bivalves[61] using liquid chromatography/electrospray ionization–tandem mass spectrometry. Tanioka *et al.*[62] have reported that B_{12} contents are considerably higher in edible bivalves (approximately 60 μg/100 g wet weight) than in edible snails (approximately 20 μg/100 g wet weight). There are three types of snails: sea, freshwater, and land snails.[63] Most snails are herbivorous, eating plants and seaweed, while some sea snails are omnivores or carnivores. The differences in the content and B_{12} compounds between these edible sea snails appear to be attributable to their dietary habitats, because ivory shell (*Babylonia japonica*; B_{12} content of meat and viscera, approximately 27.2 and 92.8 μg/100 g wet weight, respectively) and turban shell (*Turdo Batillus cornutus*; B_{12} content of meat and viscera, approximately 3.0 and 15.1 μg/100 g wet weight, respectively) are carnivorous and herbivorous sea snails, respectively.[64]

The B_{12} content (0.2–0.5 μg/100 g dry weight) of seaweeds as foods of herbivorous sea snails (turban shell, *T. cornutus*) is very low.[21] Moreover, wakame predominantly contains certain B_{12} analogues.[65] Other herbivorous sea snails (such as abalone) mainly contain pseudoB_{12}.[66] Escargot products contain a small amount (approximately 2.2 μg/100 g wet weight) of B_{12} and two inactive corrinoids, which have been identified as factor IIIm (methoxymensimidazolyl cobamide) and factor S (2-methylmercaptoadenyl cobamide) using liquid chromatography/electrospray ionization–tandem mass spectrometry.[67] In particular, 2-methylmercaptoadenyl cyanocobamide is reportedly the predominant corrinoid in human feces.[68] These results suggest that these edible bivalves and carnivorous sea snails are good sources of B_{12} for humans.

Vitamin B_{12} in plant-derived food

Most plants neither produce nor require B_{12}.[69] Methylotrophys inhabit soil, water, and plants[70,71]: in aerial surfaces of plants, *Methylobacaterium* sp. utilizes methanol emitted by plants; in aquatic environments, methanotrophys colonize macrophytic algae; and in soil, methonotrphs require B_{12} supplied from rhizobial bacteria. Furthermore, some species of *Methylobacterium* such as *Methylobacterium extroquences* NR-1[72] and the *Methylobacterium aquaticum* strain 22A[73] have B_{12} biosynthetic pathways. Thus, plant–bacterial interactions play important roles in plant growth because B_{12} deficiency inhibits plant growth under nitrogen-limited conditions.[70,74]

B_{12} has also been detected in the fruiting bodies of various mushrooms that cannot synthesize B_{12}.[75] High B_{12} was detected in mushrooms with enhanced contact with B_{12}-synthesizing bacteria in the soil,[76] suggesting that B_{12} found in mushroom fruiting bodies was derived from B_{12} sources outside the mushrooms, such as concomitant B_{12}-synthesizing bacteria.

As described above, in aquatic environments, phytoplankton–bacterial interactions play important roles in algal growth because half of all algae require B_{12}.[77] Even in phytoplankton or microalgae without the dependence of B_{12} for growth, B_{12} was absorbed, accumulated, and used as a cofactor of B_{12}-dependent methionine synthase (or MetH), which has more efficient catalytic ability than B_{12}-independent methionine synthase (or MetE)[77,78] (Figure 5).

Edible plants

Sea buckthorn (*Hippophae rhamnoides*) berries and granulate products, sidea couch grass (*Elymus repens*) products (dry extract and grinded), and elecampane (*Inula helenium*) reportedly contain considerable amounts of B_{12} (approximately 11–37 μg/100 g of dry weight),[79] suggesting that B_{12} found in these plant and plant products is due to a symbiosis with B_{12}-synthesizing bacteria.

B_{12}-enriched vegetables

Organic fertilizers such as cow manure appear to slightly increase the B_{12} content of spinach leaves (approximately 0.14 μg/100 g fresh weight).[80] Our published[81] and unpublished data have indicated that organic fertilizers mainly contain inactive corrinoids. B_{12}-enriched vegetables have been prepared by treating them with a B_{12} solution,[82,83] suggesting that free B_{12}-supplemented vegetables may be beneficial to elderly persons because the malabsorption of protein bound B_{12} is most commonly seen in the elderly.[84]

Mushroom

Trace levels (<0.1 μg/100 g dry weight) of B_{12} have been found in the dried fruiting bodies of black morels, oyster mushrooms, parasol mushrooms, and porcini mushrooms.[75] However, the fruiting bodies of black trumpet (*Craterellus cornucopioides*) and golden chanterelle (*Cantharellus cibarius*) contain slightly higher levels of B_{12} (1.09–2.65 μg/100 g dry weight).[75] In addition, the B_{12} contents of commercially available dried shiitake mushroom (*Lentinula edodes*) fruiting bodies significantly varied, with the average B_{12} value approximately 5.6 μg/100 g dry weight.[85] B_{12} found in shiitake mushroom fruiting bodies has not been attributed to the *de novo* biosynthesis of B_{12}, but appears to be derived from B_{12} sources outside the mushrooms, presumably concomitant B_{12}-synthesizing bacteria or those existing in bed logs.[85] Similarly cultivated white button mushroom (*Agaricus bisporus*) fruiting bodies contain approximately 0.2 μg of B_{12} per 100 g dry weight,[86] with the highest B_{12} content found in the peel portion. B_{12} was also detected at similar levels in their composts. These results suggest that white button mushroom can absorb B_{12} from the compost or B_{12}-synthesizing bacteria on the mushroom surface. Truffles (*Tuber* sp.) live in a close mycorrhizal association with the roots of specific host trees and their fruiting bodies grow underground. Indeed, B_{12} contents (approximately 11.5 μg of B_{12} per 100 g dry weight) of several truffle fruiting bodies are higher than those reported for other edible mushroom fruiting bodies.[76] There is no information available on the physiological function of B_{12} in these mushrooms. Dried shiitake mushroom fruiting bodies rarely contain the inactive corrinoid, B_{12}[c-lactone].[85] Lion's mane mushroom (*Hericium erinaceus*) fruiting bodies contain considerable amounts of B_{12}[c-lactone].[87] B_{12}[c-lactone] binds weakly to the intrinsic factor, which is involved in the gastrointestinal absorption of B_{12} and inhibits the B_{12}-dependent enzymes.[88] These results suggest that these mushroom fruiting bodies are not good sources of B_{12} for vegetarians because of their

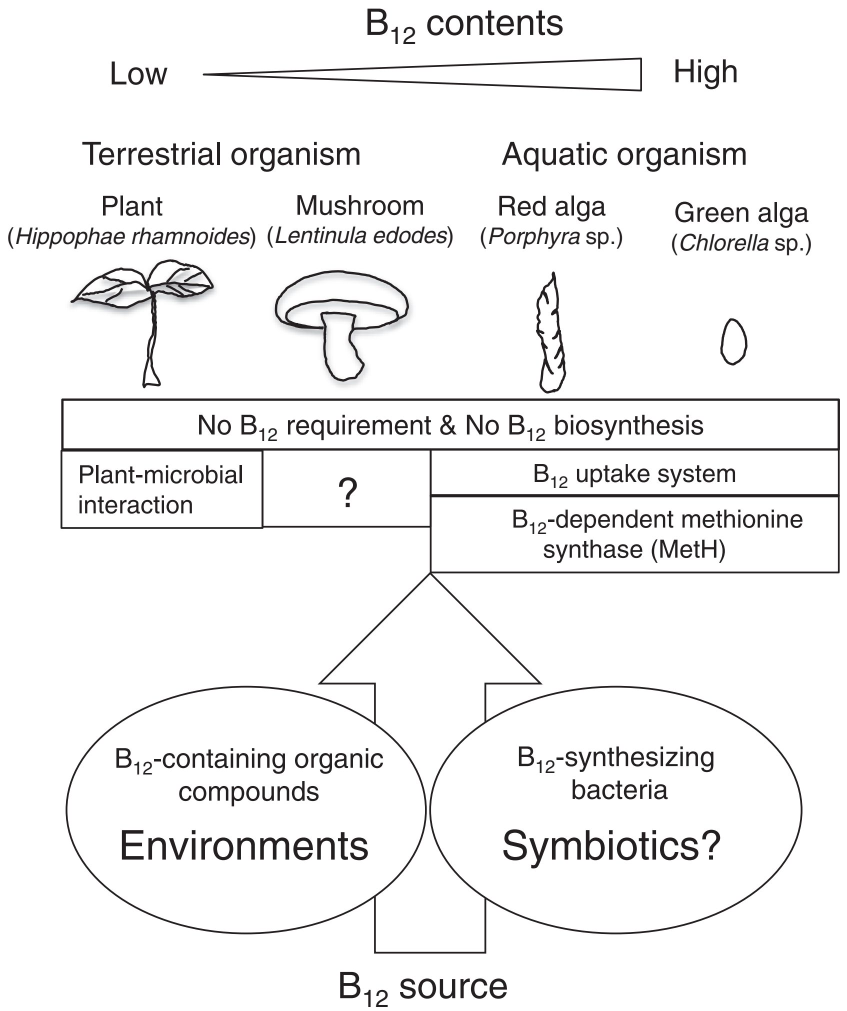

Figure 5. Vitamin B_{12} sources and microbial interactions in edible plants, mushrooms, and algae. Most plants neither produce nor require B_{12}. Methylotrophys inhabit soil, water, and plants (aerial surfaces of plants) and some of them have B_{12} biosynthetic pathways. Plant–bacterial interactions play important roles in plant growth. Mushrooms cannot synthesize B_{12}, but B_{12} found in mushroom fruiting bodies is derived from B_{12} sources outside the mushrooms, including concomitant B_{12}-synthesizing bacteria. In aquatic environments, phytoplankton–bacterial interactions play important roles in algal growth because half of all algae require B_{12}. Even in phytoplankton or microalgae without a dependence on B_{12} for growth, B_{12} accumulates and is used as a cofactor of B_{12}-dependent methionine synthase (MetH).

lower B_{12} content and the occurrence of harmful B_{12}[c-lactone] even in rare cases.

Red algae

The red algae *Porphyra* sp. is one of the most commercially available marine crops and well known as a sea vegetable.[89] Various species of *Porphyra* are widely consumed as dried nori sheet products, which contain substantial amounts of B_{12} (approximately ~77.6 µg/100 g dry weight).[90] Our results[91] and unpublished data have indicated that dried Chinese nori (zicai), dried New Zealand nori (karengo), dried Korean nori (kim), and canned Welsh nori (laverbread) contain approximately 60.2, 28.5, 66.8, and 2.8 µg of B_{12} per 100 g weight, respectively. The characterization of B_{12} compounds found in edible algae including *Porphyra* sp. have been described in the literature.[10,90] Genomic analyses of *Porphyra umbilicalis* have suggested the physiological function of B_{12} as well as evolutionary insights in red algae.[92] Our studies of naturally occurring plant-based foods with high B_{12} contents suggests that nori is the most suitable B_{12} source presently available for vegans.[93] B_{12} from dried nori is significantly absorbed and functional in B_{12}-depleted rats.[94,95]

Green algae

The green alga *Chlorella* sp. is used in human food supplements and contains biologically active B_{12}.[96–99] Recently, we analyzed B_{12} compounds in 19 dried *Chlorella* health supplements. *Chlorella* B_{12} contents varied from <0.1 µg to approximately 415 µg per 100 g of dry weight.[100] *Chlorella* cell types of the low B_{12} group were aseptically grown in large culture vessels (closed culture conditions), and the other *Chlorella* cell types were openly grown in large culture tanks (open culture conditions). Among the *Chlorella* species, B_{12} contents were much higher in *Chlorella pyrenoidosa* than in *Chlorella vulgaris* under open culture conditions.[100] *Chlorella* cells reportedly have an uptake system of exogenous B_{12}.[101] Thus, B_{12} compounds in *Chlorella* cells are likely derived from B_{12}-synthesizing bacteria that are present under open culture conditions or from the addition of crystalline B_{12} or from B_{12}-containing organic ingredients in the culture medium.

The coenzyme forms of B_{12}, 5′-deoxyadenosylcobalamin (approximately 32%) and methylcobalamin (approximately 8%), were considerably present in *Chlorella* tablets,[100] whereas cyanocobalamin was present at the lowest concentrations. *Chlorella* NC64A reportedly expresses homologous genes that encode B_{12}-dependent and -independent methionine synthases and methylmalonyl-CoA mutase.[102] Indeed, B_{12}-dependent methionine synthase and methylmalonyl-CoA mutase activities were detected in cell homogenates of *C. pyrenoidosa*.[100]

We stress that if *Chlorella* tablets are to be consumed as a sole B_{12} source, *Chlorella* tablets with moderate or high levels of B_{12} must be identified using liquid chromatography/electrospray ionization–tandem mass spectrometry, because inactive corrinoid compounds (a cobalt-free corrinoid and 5-methoxybenzimidazolyl cyanocobamide) were rarely detected in some high B_{12}-containing *Chlorella* tablets.[100]

Conclusion

B_{12} is synthesized by certain bacteria and archaeon, but not by plants or animals. The synthesized B_{12} is transferred and accumulates in animal tissues, and even in certain plant tissues *via* microbial interaction. Meats and milks of herbivorous ruminant animals are good sources of B_{12} for humans. Ruminants acquire the essential nutrient B_{12} through a symbiotic relationship with bacteria inside the body. In a broad sense, we (except vegetarians) also depend on B_{12}-producing bacteria located in ruminant stomachs. In aquatic environments, most phytoplankton acquire B_{12} through a symbiotic relationship with bacteria. Even algae that have no requirement of B_{12} for growth can accumulate substantial amounts of B_{12} and have the ability to use B_{12} as a cofactor in B_{12}-dependent methionine synthase. Then, phytoplankton becomes food for fish and bivalves in the natural food chain. Thus, humans acquire B_{12} formed by a microbial interaction *via* mainly ruminants and fish (or shellfish) as foods. Recently, it was reported that B_{12} is a modulator of gut microbial ecology.[103] The bioavailability of food B_{12} is approximately 50% in healthy humans[104] and unabsorbed B_{12} would affect intestinal microbial ecology, which is expected to have a substantial impact on human health.

Author contributions: All authors contributed equally to the preparation of this manuscript and have approved the final version.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article

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