

MINIREVIEW

Vitamin B₁₂ Sources and Bioavailability

FUMIO WATANABE¹

*School of Agricultural, Biological and Environmental Sciences, Faculty of Agriculture,
Tottori University, Tottori 680-8553, Japan*

The usual dietary sources of vitamin B₁₂ are animal foods, meat, milk, egg, fish, and shellfish. As the intrinsic factor-mediated intestinal absorption system is estimated to be saturated at about 1.5–2.0 µg per meal under physiologic conditions, vitamin B₁₂ bioavailability significantly decreases with increasing intake of vitamin B₁₂ per meal. The bioavailability of vitamin B₁₂ in healthy humans from fish meat, sheep meat, and chicken meat averaged 42%, 56%–89%, and 61%–66%, respectively. Vitamin B₁₂ in eggs seems to be poorly absorbed (<9%) relative to other animal food products. In the Dietary Reference Intakes in the United States and Japan, it is assumed that 50% of dietary vitamin B₁₂ is absorbed by healthy adults with normal gastrointestinal function. Some plant foods, dried green and purple lavers (nori) contain substantial amounts of vitamin B₁₂, although other edible algae contained none or only traces of vitamin B₁₂. Most of the edible blue-green algae (cyanobacteria) used for human supplements predominately contain pseudovitamin B₁₂, which is inactive in humans. The edible cyanobacteria are not suitable for use as vitamin B₁₂ sources, especially in vegans. Fortified breakfast cereals are a particularly valuable source of vitamin B₁₂ for vegans and elderly people. Production of some vitamin B₁₂-enriched vegetables is also being devised. *Exp Biol Med* 232:1266–1274, 2007

Key words: vitamin B₁₂; cobalamin; food source; bioavailability; deficiency; human

Vitamin B₁₂ is the largest (molecular weight = 1355.4) and most complex of all the vitamins. Although the scientific use of the term “vitamin

B₁₂” is usually restricted to cyanocobalamin, vitamin B₁₂ represents all potentially biologically active cobalamins in this review. Cobalamin is the term used to refer to a group of cobalt-containing compounds (corrinoids) that have a lower axial ligand that contains the cobalt-coordinated nucleotide (5, 6-dimethylbenzimidazole as a base; Fig. 1). Cyanocobalamin, which is used in most supplements, is readily converted to the coenzyme forms of cobalamin (methylcobalamin and 5'-deoxyadenosylcobalamin) in the human body (1).

Vitamin B₁₂ is synthesized only in certain bacteria (2). The vitamin B₁₂ synthesized by bacteria is concentrated mainly in the bodies of higher predatory organisms in the natural food chain system. Animal foods (i.e., meat, milk, egg, fish, and shellfish) but not plant foods are considered to be the major dietary sources of vitamin B₁₂ (1). Some plant foods, such as edible algae or blue-green algae (cyanobacteria), however, contain large amounts of vitamin B₁₂. Vitamin B₁₂ compounds in algae appear to be inactive in mammals (3). Foods contain various vitamin B₁₂ compounds with different upper ligands; methylcobalamin and 5'-deoxyadenosylcobalamin function, respectively, as coenzymes of methionine synthase (EC 2.1.1.13), which is involved in methionine biosynthesis and of methylmalonyl-CoA mutase (EC 5.4.99.2), which is involved in amino acid and odd-chain fatty acid metabolism in mammalian cells (4, 5).

Humans have a complex process for gastrointestinal absorption of dietary vitamin B₁₂ (6). Vitamin B₁₂ released from food protein is first bound to haptocorrin (salivary vitamin B₁₂-binding protein) in the stomach. After proteolysis of haptocorrin-vitamin B₁₂ complex by pancreatic proteases in the duodenum, the released vitamin B₁₂ binds to intrinsic factor (IF, gastric vitamin B₁₂-binding protein) in the proximal ileum. The IF-vitamin B₁₂ complex can enter mucosal cells in the distal ileum by receptor-mediated endocytosis. Bioavailability of dietary vitamin B₁₂ is significantly dependent on this gastrointestinal absorption

This research was supported in part by a fund for Comprehensive Research on Cardiovascular Diseases from The Ministry of Health, Labor, and Welfare of Japan.

¹ To whom correspondence should be addressed at School of Agricultural, Biological and Environmental Sciences, Faculty of Agriculture, Tottori University, 4-101 Koyama-Minami, Tottori 680-8553, Japan. E-mail: watanabe@muses.tottori-u.ac.jp

DOI: 10.3181/0703-MR-67
1535-3702/07/23210-1266\$15.00
Copyright © 2007 by the Society for Experimental Biology and Medicine

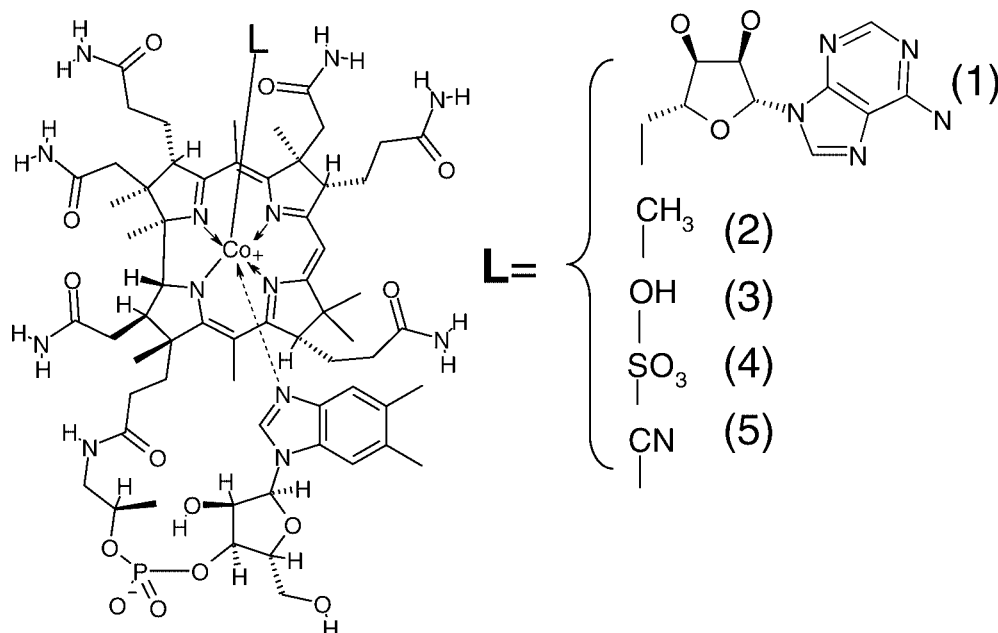


Figure 1. Structural formula of vitamin B₁₂ and partial structures of vitamin B₁₂ compounds. The partial structures of vitamin B₁₂ compounds show only those portions of the molecule that differ from vitamin B₁₂. 1: 5'-deoxyadenosylcobalamin; 2, methylcobalamin; 3, hydroxocobalamin; 4, sulfitecobalamin; 5, cyanocobalamin or vitamin B₁₂.

system. In the Dietary Reference Intakes in the United States, it is assumed that 50% of dietary vitamin B₁₂ is absorbed by healthy adults (7); however, there are few data on the bioavailability of vitamin B₁₂ from foods. In this article presented here, up-to-date information is reviewed on vitamin B₁₂ content and bioavailability in various foods in relation to the prevention of vitamin B₁₂ deficiency.

Requirements of Vitamin B₁₂ and Vitamin B₁₂ Deficiency

The major signs of vitamin B₁₂ deficiency are megaloblastic anemia and neuropathy (7). Strict vegetarians (vegans) have a greater risk of developing vitamin B₁₂ deficiency relative to nonvegetarians (8) and must consume vitamin B₁₂-fortified foods or vitamin B₁₂-containing dietary supplements to prevent vitamin B₁₂ deficiency. A considerable proportion of elderly subjects having low serum vitamin B₁₂ levels without pernicious anemia have been reported to have malabsorption of protein-bound vitamin B₁₂ (food-bound vitamin B₁₂ malabsorption; Ref. 9). The food-bound vitamin B₁₂ malabsorption is found in patients with certain gastric dysfunctions, such as atrophic gastritis with decreased stomach acid secretion (10). Because the bioavailability of crystalline vitamin B₁₂ is not altered in patients with atrophic gastritis, the Institute of Medicine recommended that adults 51 years and older should obtain the majority of the recommended dietary allowance (RDA) of vitamin B₁₂ through the consumption of foods fortified with crystalline vitamin B₁₂ or vitamin B₁₂-containing supplements (7). Seal *et al.* (11) reported

that a slightly higher dose (50 µg/day) of vitamin B₁₂ supplementation significantly increases serum vitamin B₁₂ concentrations in older patients with subnormal vitamin B₁₂ status.

The RDA of vitamin B₁₂ for adults is set at 2.4 µg/day in the United States (and Japan); however, daily body loss of the vitamin is estimated to be between 2 and 5 µg/day (7). Bor *et al.* (12) reported that a daily vitamin B₁₂ intake of 6 µg appears to be sufficient to maintain a steady-state concentration of plasma vitamin B₁₂ and vitamin B₁₂-related metabolic markers.

Assay of Vitamin B₁₂ in Foods

Historically, vitamin B₁₂ content of foods has been determined by bioassay with certain vitamin B₁₂-requiring microorganisms, such as *Lactobacillus delbrueckii* subsp. *lactis* ATCC7830 (formerly *Lactobacillus leichmannii*; Ref. 13). Radioisotope dilution assay (RIDA) method with radiolabeled vitamin B₁₂ and hog IF (the most specific vitamin B₁₂-binding protein) has also been used for the determination of vitamin B₁₂ content in foods (14). Although it was reported that the values determined by the RIDA method were slightly higher in human serum than those determined by the microbiologic method (15), Casey *et al.* (14) demonstrated the excellent correlation between both methods in food vitamin B₁₂ analysis.

A fully automated chemiluminescence vitamin B₁₂ analyzer with the acridinium ester-labeled vitamin B₁₂ derivative and IF has been commercialized. Currently, various types of similar vitamin B₁₂ analyzers are being

manufactured and clinically used for the routine assay of human serum vitamin B₁₂ worldwide. About 10 years ago, my colleagues and I evaluated the applicability of this machine in food analysis, indicating the excellent correlation coefficient between both methods in most foods tested, although in some specific foods the values determined by the microbiologic method were about several-fold greater than the values determined by the chemiluminescence method (16). This difference may be due to the fact that *L. delbrueckii* used for the microbiologic assay of food vitamin B₁₂ uses corrinoid compounds that are inactive for humans as well as vitamin B₁₂. Ball (1) stated that about 30% of the reported vitamin B₁₂ in foods may be microbiologically active corrinoids rather than vitamin B₁₂. Furthermore, it is known that both deoxyribosides and deoxynucleotides (known as the alkali-resistant factor) can substitute vitamin B₁₂ in this lactic bacterium (17).

Vitamin B₁₂ in Animal Food

Meat. In the United States Department of Agriculture database, vitamin B₁₂ contents of cooked beef liver, lean meat, and turkey are estimated to be 83, 3, and 33 µg/100 g, respectively (18). Appreciable losses (~33%) of vitamin B₁₂ in meats by cooking have been reported (19, 20).

Bioavailability of vitamin B₁₂ from 100 g (0.9 µg vitamin B₁₂), 200 g (3.0 µg), and 300 g (5.1 µg) of ground patties cooked from mutton (labeled with radioactive vitamin B₁₂) in normal human subjects averaged 56%–77%, 76%–89%, and 40%–63%, respectively (21). An average absorption of vitamin B₁₂ from liver pâté (38 µg vitamin B₁₂) is approximately 10%. Since the IF-mediated intestinal absorption system is estimated to be saturated at about 1.5–2.0 µg per meal under the physiologic conditions (22), vitamin B₁₂ bioavailability should decrease significantly with increases in the intake of vitamin B₁₂ per meal.

Absorption of vitamin B₁₂, assessed by measuring fecal excretion of radioactivity, after consuming 100 g (0.4–0.6 µg vitamin B₁₂), 200 g (0.8–1.3 µg), and 300 g (1.3–1.9 µg) of chicken meat (labeled with radioactive vitamin B₁₂) in healthy human subjects averaged 65%, 63%, and 61%, respectively (23).

Milk. Although vitamin B₁₂ content (0.3–0.4 µg/100 g) of various types of milk is not high (18), milk and dairy products are significant contributors of vitamin B₁₂ intakes, since the intake of dairy products is high in the general population (7). In bovine milk, all naturally occurring vitamin B₁₂ is bound to the transcobalamin, one of the mammalian vitamin B₁₂-binding proteins (24). When radioactive vitamin B₁₂ (0.25 µg) mixed in water or milk was administered to human subjects, the mean absorption, as assessed by a whole-body counting of radioactivity, was 55% or 65%, respectively (25).

Appreciable losses of vitamin B₁₂ have been reported during the processing of milk; boiling for 2–5 min and 30 min resulted in 30% and 50% loss, respectively (1, 20). The

5-min microwave cooking led to 50% loss and 5%–10% lost by pasteurization (1, 20). When various milk samples were exposed to fluorescent light for 24 hrs at 4°C, the vitamin B₁₂ concentration decreased considerably, depending on the type of milk tested (26). On the other hand, when the pasteurized milk was refrigerated for 9 days under retail-simulating or domestic handling conditions, there was no appreciable decline in the concentration of milk vitamin B₁₂ (27).

Vitamin B₁₂ concentrations in fermented milk decreased significantly during storage at 4°C for 14 days relative to the original milk. About 20%–60% of vitamin B₁₂ that is originally presented in milk is recovered in cottage cheese, hard cheese, and blue cheese (28). Sato *et al.* (29) demonstrated that the content of vitamin B₁₂ in the whey is reduced considerably during lactic acid fermentation. This decrease in vitamin B₁₂ content in whey is due to the production of vitamin B₁₂ compounds that are not easily extracted for detection by conventional extraction method. Although the vitamin B₁₂ compounds could be extracted by sonication and treatment by proteases, such as pepsin and papain, no information is available on any chemical properties of these compounds (29).

Egg. Vitamin B₁₂ content in the whole egg is about 0.9–1.4 µg/100 g (18, 30), and most of the vitamin B₁₂ is found in the egg yolk (31). Vitamin B₁₂ intakes from the egg are generally large, because it is a popular food item (7). Bioavailability of vitamin B₁₂ from scrambled egg yolks, scrambled whole eggs, boiled eggs, and fried eggs (1.1–1.4 µg vitamin B₁₂ per 100 g) averaged 8.2%, 3.7%, 8.9%, and 9.2%, respectively (30). Vitamin B₁₂ in eggs is generally poorly absorbed relative to other animal food products (32).

Shellfish. Various shellfish are consumed widely. The shellfish that siphon large quantities of vitamin B₁₂-synthesizing microorganisms in the sea are known to be excellent sources of vitamin B₁₂, of which concentrations can exceed sometimes 10 µg/100 g (33). The vitamin B₁₂-synthesizing microorganisms can also synthesize various corrinoids (including corrinoid compounds inactive for humans) with different bases in the lower ligand. When corrinoid compounds were isolated and characterized in popular shellfish, such as oysters, mussels, and short-necked clams, each corrinoid compound was identified as vitamin B₁₂ (34). The higher values in the determination of vitamin B₁₂ by the microbiologic method compared with the chemiluminescence method may be due to occurrence of certain vitamin B₁₂-substitutive compounds, of which chemical properties have not been characterized.

Fish. Fish (or shellfish) contribute greatly to vitamin B₁₂ intake among Asians, particularly Japanese people, and this trend is spreading throughout the world (35). In the USDA database, vitamin B₁₂ contents of certain fish (salmon, sardine, trout, tuna, etc.) are 3.0 to 8.9 µg/100 g (18). Based on our studies, the dark muscle of skipjack contains a substantial amount (159 µg/100 g) of vitamin B₁₂ compared with the light muscle (dorsal portion 10 µg/100 g;

ventral portion 8 µg/100 g; Ref. 36). When a corrinoid compound was isolated and characterized in the dark muscle, it was identified as vitamin B₁₂. Similar results of high vitamin B₁₂ content in dark muscle were found in the yellowfin tuna (37).

Various commercially available soup stocks, which are mainly made of dried bonito shavings and dried sardines, contain considerable amounts (0.2 to 1.2 µg/100 ml) of free vitamin B₁₂, indicating that these may be excellent free vitamin B₁₂ sources.¹ The loss of vitamin B₁₂ from fish meat by various cooking methods (boiling, steaming, sautéing, frying, and microwaving) was not high, with a range of 2.3%–14.8% (36).

Doscherholmen *et al.* (38) measured the bioavailability of radioactive vitamin B₁₂ that was injected into the rainbow trout. A few weeks after this injection, the bioavailability of vitamin B₁₂ from the fish meat was evaluated. The bioavailabilities of labeled vitamin B₁₂ in 50 g (equivalent to 2.1 µg vitamin B₁₂), 100 g (4.1 µg), 200 g (9.2 µg), and 300 g (13.3 µg) of fish meat were 42%, 38%, 42%, and 30%, respectively.

Salted and Fermented Fish. The highest amount of vitamin B₁₂ among foods described in the Japanese Standard Tables of Food Composition is 328 µg/100 g in salted and fermented salmon kidney that is called “Mefun” (39). Eating only 0.8 g Mefun can supply the total RDA (2.4 µg/day) for the adult population. Although this item has a delicate flavor, it has an extremely limited application, since it is popular only in Japan. It might be interesting, however, to describe the characterization of vitamin B₁₂ in this item, which may potentially have the highest vitamin B₁₂ content in nature. The vitamin B₁₂ found in Mefun is not derived from concomitant vitamin B₁₂-synthesizing bacteria, but is accumulated in the salmon kidney. The majority of vitamin B₁₂ found in Mefun was recovered in the free vitamin B₁₂ fractions (40). Mefun may be an excellent free vitamin B₁₂ source for elderly subjects with food-bound vitamin B₁₂ malabsorption.

Fish Sauce. Various kinds of fish sauces, traditional food supplements in the diet, are widely used as a seasoning worldwide. Fish sauce (Nam-pla) appears to constitute a major source of vitamin B₁₂ in Thailand, since it contains considerable amounts of vitamin B₁₂ (41). A considerable amount of vitamin B₁₂ (range: 2.3 to 5.5 µg/100 g) was also found in “Ishiru” (a Japanese traditional fish sauce; Ref. 42). When two corrinoid compounds in the fish sauce were isolated and characterized, the main compound was identified as vitamin B₁₂, but the other minor compound could not be identified (42). Corrinoid compounds found in various fish sauces made in Japan could not be identified (43). Fish sauce may not be suitable for use as a source of vitamin B₁₂, considering the low daily intake of the sauce and occurrence of the unidentified corrinoid compounds.

Vitamin B₁₂ in Plant Food

Vegetables. Many studies have been performed to measure vitamin B₁₂ content in various vegetables. For decades, edible bamboo shoots have been believed to contain considerable amounts of vitamin B₁₂. However, it turned out that they do not contain appreciable amounts of vitamin B₁₂; however, certain compounds showing vitamin B₁₂-like activity (known as the alkali-resistant factor) were found in them (44). Similar results were found in cabbage, spinach, celery, garland chrysanthemum, lily bulb, and taro (44). Only trace amounts of vitamin B₁₂ (<0.1 µg/100 g of wet weight edible portion), which was estimated by subtracting the alkali-resistant factor from total vitamin B₁₂, were found in broccoli, asparagus, Japanese butterbur, mung bean sprouts, tassa jute, and water shield (44). These vegetables may have the ability to take up vitamin B₁₂ found in certain organic fertilizer.

Mozafar (45) demonstrated that the addition of an organic fertilizer, cow manure, significantly increases the vitamin B₁₂ content in barley kernels and spinach leaves. Mozafar and Oeftli (46) investigated uptake of vitamin B₁₂ by soybean roots under water culture conditions. Sato *et al.* (47) reported that a high level of vitamin B₁₂ is incorporated into a vegetable, kaiware daikon (radish sprout), by soaking its seeds in vitamin B₁₂ solutions before germination. The amount of vitamin B₁₂ incorporated into kaiware daikon increases up to about 170 µg/100 g of wet sprout with 3-hr soaking of seeds in 200 µg/ml vitamin B₁₂ solution. These vitamin B₁₂-enriched vegetables may be of special benefit to vegans or elderly persons with food-bound vitamin B₁₂ malabsorption.

Tea Leaves and Tea Drinks. Considerable amounts of vitamin B₁₂ are found in various types of tea leaves: green (0.1–0.5 µg vitamin B₁₂ per 100 g dry weight), blue (about 0.5 µg), red (about 0.7 µg), and black (0.3–1.2 µg) tea leaves (48).

When a corrinoid compound was isolated from Japanese fermented black tea (Batabata-cha), the compound was identified as vitamin B₁₂ (49). When vitamin B₁₂-deficient rats were fed this tea drink (50 ml/day, equivalent to a daily dose of 1 ng vitamin B₁₂) for 6 weeks, urinary methylmalonic acid excretion (an index of vitamin B₁₂ deficiency) of the tea drink-supplemented rats decreased significantly compared with that of the deficient rats (49). These results indicate that the vitamin B₁₂ found in the fermented black tea is bioavailable in rats. However, only 1–2 liters of consumption of fermented tea drink (typical regular consumption in Japan), which is equivalent to 20–40 ng vitamin B₁₂, is not sufficient to meet the RDA of 2.4 µg/day for adult humans.

Soybean. Vitamin B₁₂ contents of soybean are low or undetectable. A soybean-fermented food, tempe, contains a large amount of vitamin B₁₂ (0.7 to 8 µg/100 g; Ref. 50). Certain bacteria contamination during the process of tempe production may contribute to the vitamin B₁₂ increase of

¹Nishioka M, Miyamoto E, and Watanabe F. Unpublished data.

tempe (51). Another fermented soybean, natto, contains a minute amount of vitamin B₁₂ (0.1 to 1.5 µg/100 g; Ref. 52).

Edible Algae. Various types of edible algae are used for human consumption the world over. Dried green (*Enteromorpha* sp.) and purple (*Porphyra* sp.) lavers (nori) are the most widely consumed among the edible algae and contain substantial amounts of vitamin B₁₂ (32 to 78 µg/100 g dry weight; Ref. 39). In Japanese cooking, several sheets of nori (9 × 3 cm; about 0.3 g each) are often served for breakfast. A large amount of nori (<6 g) can be consumed from certain sushi, vinegared rice rolled in nori. However, edible algae other than these two species contain none or only traces of vitamin B₁₂ (39). Dagnelie *et al.* (53) reported the effect of edible algae on the hematologic status of vitamin B₁₂-deficient children, suggesting that algal vitamin B₁₂ appears to be nonbioavailable. As bioavailability of the algal vitamin B₁₂ is not clear in humans, my colleagues and I characterized corrinoid compounds to determine whether the dried purple and green lavers and eukaryotic microalgae (*Chlorella* sp. and *Pleurochrysis carterae*) used for human food supplements contain vitamin B₁₂ or inactive corrinoids. My colleagues and I found that these edible algae contain a large amount of vitamin B₁₂ without the presence of inactive corrinoids (54–57).

To measure the bioavailability of vitamin B₁₂ in the lyophilized purple laver (*Porphyra yezoensis*), the effects of feeding the laver on various parameters of vitamin B₁₂ were investigated in vitamin B₁₂-deficient rats (58). Within 20 days after vitamin B₁₂-deficient rats were fed a diet supplemented with dried purple laver (10 µg vitamin B₁₂/kg diet), urinary methylmalonic acid excretion became undetectable and hepatic vitamin B₁₂ (especially coenzyme vitamin B₁₂) levels significantly increased. These results indicate that vitamin B₁₂ from the purple lavers is bioavailable in rats.

A nutritional analysis for the dietary food intake and serum vitamin B₁₂ level of a group of six vegan children aged 7 to 14 who had been living on a vegan diet including brown rice for 4 to 10 years suggests that consumption of nori may keep vegans from suffering vitamin B₁₂ deficiency (59). Rauma *et al.* (60) also reported that vegans consuming nori and/or chlorella had a serum vitamin B₁₂ concentration twice as high as those not consuming these algae.

Edible Cyanobacteria. Some species of the cyanobacteria, including *Spirulina*, *Aphanizomenon*, and *Nostoc*, are produced at annual rates of 500–3000 tons for food and pharmaceutical industries worldwide (61). Tablets containing *Spirulina* sp. are sold as a health food fad, since it is known to contain a large amount of vitamin B₁₂ (62). We found that commercially available spirulina tablets contained 127–244 µg vitamin B₁₂ per 100 g weight (63). When two corrinoid compounds were characterized from the spirulina tablets, the major (83%) and minor (17%) compounds were identified as pseudovitamin B₁₂ (adeninly cobamide) and vitamin B₁₂, respectively (Fig. 2). Several

groups of investigators indicated that pseudovitamin B₁₂ is hardly absorbed in mammalian intestine with a low affinity to IF (64, 65). Furthermore, researchers showed that spirulina vitamin B₁₂ may not be bioavailable in mammals (63, 66). Herbert (67) reported that an extract of spirulina contains two vitamin B₁₂ compounds that can block the metabolism of vitamin B₁₂. And van den Berg *et al.* (68) demonstrated that a spirulina-supplemented diet does not induce severe vitamin B₁₂ deficiency in rats, implying that the feeding of spirulina may not interfere with the vitamin B₁₂ metabolism. Further studies are needed to clarify bioavailability of spirulina vitamin B₁₂ in humans.

Aphanizomenon flos-aquae, a fresh water cyanobacterium, grow naturally in Upper Klamath Lake, Oregon. Kay (69) described that the bacterial cells contain some corrinoid compounds that can be used as vitamin B₁₂ in humans. In contrast, my colleagues and I found that the corrinoid compound purified from *Aphanizomenon* cells was identified as pseudovitamin B₁₂, which is inactive corrinoid for humans (70). We found that the dried bacterial cells contained 616 µg vitamin B₁₂ per 100 g weight. *Escherichia coli* 215-bioautography of the *Aphanizomenon* extract indicated that the bacterial cells contained only pseudovitamin B₁₂ (70).

Aphanothece sacrum (Suizenji-nori) is an edible cyanobacterium that is indigenous to Japan. The dried bacterial cells are used as an ordinary food item after soaking in water or a nutritional supplement. The nutrition labeling of this bacterial product shows that the dried cells contain a large amount of vitamin B₁₂ (94 µg/100 g); however, the corrinoid compound purified from the bacterial cells was identified as pseudovitamin B₁₂ (71). Therefore, its nutritional value is questionable. *Nostoc commune* (Ishikurage) contains considerable amounts (99 µg/100 g) of vitamin B₁₂ in its dried cells as measured by the microbiologic method; however, only 12% of the vitamin may be active, since the main (88%) and minor (12%) compounds in the bacterial cells were identified as pseudovitamin B₁₂ and vitamin B₁₂, respectively (72). In summary, the results reviewed above indicate that edible cyanobacteria often contain a large amount of pseudovitamin B₁₂, which is known to be biologically inactive in humans. Therefore, they are not suitable for use as a source of vitamin B₁₂ for the prevention of vitamin B₁₂ deficiency among the high-risk population, such as vegans and elderly subjects.

Vitamin B₁₂–Fortified Cereals. Ready-to-eat cereals fortified with vitamin B₁₂ are known to constitute a great proportion of dietary vitamin B₁₂ intake in the United States (7). Several groups of investigators suggested that eating a breakfast cereal fortified with folic acid, vitamin B₁₂, and vitamin B₆ increases blood concentrations of these vitamins and decreases plasma total homocysteine concentrations in elderly populations (73). Fortified breakfast cereals have become a particularly valuable source of vitamin B₁₂ for vegetarians and elderly people.

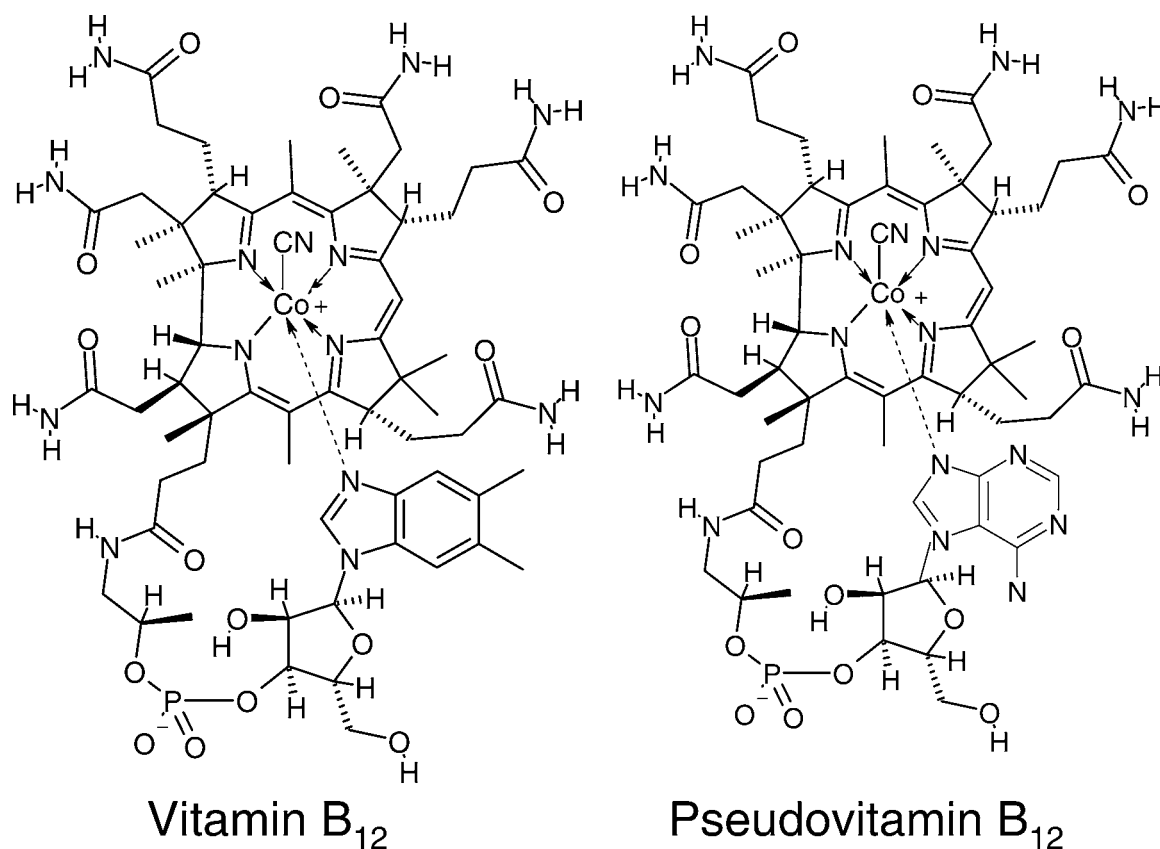


Figure 2. Structural formula of vitamin B₁₂ and pseudovitamin B₁₂ (7-adeninyl cyanocobamide).

Conclusion

Vitamin B₁₂ contents determined by the microbiologic assay method used widely in food analysis are incorrect in some specific foods, because this lactic bacterium can utilize

inactive corrinoid compounds, such as pseudovitamin B₁₂, and substitute both deoxyribosides and deoxynucleotides (known as the alkali-resistant factor) for vitamin B₁₂. Thus, vitamin B₁₂ contents should be calculated by subtracting the

Table 1. Bioavailability of Dietary Vitamin B₁₂^a

Foods	Predominate corrinoid ^b	Bioavailability ^c	Content (μg/100 g)
Animal meats			
Mutton, cooked		56%–89% (21)	2.6 (18)
Chicken, cooked		61%–66% (23)	9.4 (18)
Cow's milk		65% (25)	0.4 (18)
Eggs			
Chicken, cooked		<9% (31)	1.3 (18)
Shellfish			
Oyster	Vitamin B ₁₂ (34)		46.3 (34), 28.1 (17)
Mussel	Vitamin B ₁₂ (34)		15.7 (34), 10.3 (17)
Short-necked clam	Vitamin B ₁₂ (34)		37.0 (34), 52.4 (17)
Fish meats			
Skipjack, dark muscle	Vitamin B ₁₂ (36)		158.5 (36)
Yellowfin tuna, dark muscle	Vitamin B ₁₂ (37)		52.9 (37)
Rainbow trout, cooked		42.0% (38)	4.9 (18)
Edible algae			
Purple laver	Vitamin B ₁₂ (54)		32.3 (54), 77.6 (17)
Green laver	Vitamin B ₁₂ (55)		63.6 (55), 31.8 (17)
Chlorella	Vitamin B ₁₂ (56)		200.9–211.6 (56)

^a Numbers in parentheses are reference numbers.

^b Isolated and identified.

^c intake of <2 μg vitamin B₁₂ per meal in healthy humans.

values of the alkali-resistant factor from the values of total (or apparent) vitamin B₁₂ in all foods tested to prevent overestimating their vitamin B₁₂ contents. Even if IF-based clinical assay kits or analyzers are used for measuring food vitamin B₁₂ content, they may not represent only vitamin B₁₂ because of the possibility that the binding of vitamin B₁₂ to IF is interfered slightly by certain food ingredients or inactive corrinoid compounds, such as pseudovitamin B₁₂. The difficulty to evaluate whether certain foods contain vitamin B₁₂ or inactive corrinoids may be easily resolved by the use of a simple technique, bioautography with vitamin B₁₂-dependent *E. coli* 215 after separation of the sample by silica gel 60 thin-layer chromatography (72, 74). The database of vitamin B₁₂ content in foods should be revised in order to accurately assess dietary intakes of vitamin B₁₂.

Although food items that contribute to the vitamin B₁₂ intake vary widely depending on food cultures or food habits throughout the world, animal products (meat, milk, egg, fish, and shellfish) are excellent sources of vitamin B₁₂ (Table 1). Dried edible cyanobacteria as nutritional supplements may not be suitable for vitamin B₁₂ sources, because the majority of the vitamin in the bacterial cells is pseudovitamin B₁₂. As technologies advance, various plant foods that contain an appreciable amount of naturally occurring vitamin B₁₂ and are fortified with crystalline vitamin B₁₂ may be available for human consumption to maintain adequate vitamin B₁₂ status in the general population and to prevent vitamin B₁₂ deficiency among vegans or elderly persons.

For the Dietary Reference Intakes in the United States and Japan, it is assumed that 50% of dietary vitamin B₁₂ is absorbed and utilized by healthy adults with a normal gastrointestinal function. Further information on bioavailability of vitamin B₁₂ from various food sources of vitamin B₁₂ is needed to determine more precise RDA of the vitamin.

- Ball GFM. Vitamin B₁₂ In: Bioavailability and Analysis of Vitamins in Foods. London: Chapman & Hall, pp497–515, 1998.
- Scheider Z, Stroiński A. Biosynthesis of vitamin B₁₂. In: Schneider Z, Stroiński A, Eds. Comprehensive B₁₂. Berlin: Walter de Gruyter, pp93–110, 1987.
- Watanabe F, Takenaka S, Kittaka-Katsura H, Ebara S, Miyamoto E. Characterization and bioavailability of vitamin B₁₂-compounds from edible algae. J Nutr Sci Vitaminol 48:325–331, 2002.
- Chen Z, Crippen K, Gulati S, Banerjee R. Purification and kinetic mechanism of a mammalian methionine synthase from pig liver. J Biol Chem 269:27193–27197, 1994.
- Fenton WA, Hack AM, Willard HF, Gertler A, Rosenberg LE. Purification and properties of methylmalonyl coenzyme A mutase from human liver. Arch Biochem 228:323–329, 1982.
- Russell-Jones GJ, Aplers DH. Vitamin B₁₂ transporters. Pharm Biotechnol 12:493–520, 1999.
- Institute of Medicine. Vitamin B₁₂ In: Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B₆, Folate, Vitamin B₁₂, Pantothenic Acid, Biotin, and Choline. Washington, DC: Institute of Medicine, National Academy Press, pp306–356, 1998.
- Millet P, Guillard JC, Fuchs F, Klepping J. Nutrient intake and vitamin status of healthy French vegetarians and nonvegetarians. Am J Clin Nutr 50:718–727, 1989.
- Baik HW, Russell RM. Vitamin B₁₂ deficiency in the elderly. Annu Rev Nutr 19:357–377, 1999.
- Park S, Johnson MA. What is an adequate dose of oral vitamin B-12 in older people with poor vitamin B-12 status? Nutr Rev 64:373–378, 2006.
- Seal EC, Metz J, Flicker L, Melny J. A randomized, double-blind, placebo-controlled study of oral vitamin B₁₂ supplementation in older patients with subnormal or borderline serum vitamin B₁₂ concentrations. J Am Geriatr Soc 50:146–151, 2002.
- Bor MV, Lydeking-Olesen E, Møller J, Nexø E. A daily intake of approximately 6 µg vitamin B-12 appears to saturate all the vitamin B-12-related variables in Danish postmenopausal women. Am J Clin Nutr 83:52–58, 2006.
- Schneider Z. Purification and estimation of vitamin B₁₂. In: Schneider Z, Stroiński A, Eds. Comprehensive B₁₂. Berlin: Walter de Gruyter, pp111–155, 1987.
- Casey PJ, Speckman KR, Ebert FJ, Hobbs WE. Radioisotope dilution technique for determination of vitamin B₁₂ in foods. J Assoc Off Anal Chem 65:85–88, 1982.
- Arnaud J, Cotisson A, Meffre G, Bourgeat-Causse M, Augert C, Favier A, Vuillez JP, Ville G. Comparison of three commercial kits and a microbiological assay for the determination of vitamin B₁₂ in serum. Scand J Clin Invest 54:235–240, 1994.
- Watanabe F, Takenaka S, Abe K, Tamura Y, Nakano Y. Comparison of a microbiological assay and a fully automated chemiluminescent system for the determination of vitamin B₁₂ in food. J Agric Food Chem 46:1433–1436, 1998.
- Resources Council, Science and Technology Agency. In: Standard Tables of Food Composition in Japan-Vitamin K, B₆ and B₁₂. Tokyo: Resources Council, Science, and Technology Agency, Japan, pp6–56, 1995.
- USDA National Nutrient Database for Standard Reference, Release 18. Reports by single nutrients. Vitamin B-12 (µg) content of selected foods per common measure, sorted by nutrient content. USDA Nutrient Data Laboratory. Available at: <http://www.ars.usda.gov/services/docs.htm?docid=9673>, 2007.
- Bennink MR, Ono K. Vitamin B₁₂, E and D content of raw and cooked beef. J Food Sci 47:1786–1792, 1982.
- Watanabe F, Abe K, Fujita T, Goto M, Hiemori M, Nakano Y. Effects of microwave heating on the loss of vitamin B₁₂ in foods. J Agric Food Chem 46:206–210, 1998.
- Heyssel RM, Bozian RC, Darby WJ, Bell MC. Vitamin B-12 turnover in man. The assimilation of vitamin B-12 from natural foodstuff by man and estimates of minimal daily requirements. Am J Clin Nutr 18:176–184, 1966.
- Scott JM. Bioavailability of vitamin B₁₂. Eur J Clin Nutr 51(Suppl 1): S49–S53, 1997.
- Doscherholmen A, McMahon J, Ripley D. Vitamin B-12 assimilation from chicken meat. Am J Clin Nutr 31:825–830, 1978.
- Fedosov SN, Petersen TE, Nexø E. Transcobalamin from cow milk: isolation and physico-chemical properties. Biochim Biophys Acta 292: 113–119, 1996.
- Russell RM, Baik H, Kehayias JJ. Older man and women efficiently absorb vitamin B-12 from milk and fortified bread. J Nutr 131:291–293, 2001.
- Watanabe F, Katsura H, Abe K, Nakano Y. Effect of light-induced riboflavin degradation on the loss of cobalamin in milk. J Home Econ Jpn 51:231–234, 2000.
- Andersson I, Öste R. Nutritional quality of pasteurized milk. Vitamin B₁₂, folate and ascorbic acid content during storage. Int Dairy J 4:161–172, 1994.
- Arkbåge K, Withöft C, Fondén R, Jägerstad M. Retention of vitamin

- B₁₂ during manufacture of six fermented dairy products using a validated radio protein-binding assay. *Int Dairy J* 13:101–109, 2003.
29. Sato K, Wang X, Mizoguchi K. A modified form of a vitamin B-12 compound extracted from whey fermented by *Lactobacillus helveticus*. *J Dairy Sci* 80:2701–2705, 1997.
 30. Squires MW, Naber EC. Vitamin profiles of eggs as indicators of nutritional status in the laying hen: vitamin B₁₂ study. *Poul Sci* 71:275–282, 1992.
 31. Doscherholmen A, McMahon J, Ripley D. Vitamin B₁₂ absorption from eggs. *Proc Soc Exp Biol Med* 149:987–990, 1975.
 32. Doscherholmen A, McMahon J, Ripley D. Inhibitory effect of eggs on vitamin B-12 absorption: description of a simple ovalbumin ⁵⁷Co-vitamin B₁₂ absorption test. *Br J Haematol* 33:261–272, 1976.
 33. Herbert V. Vitamin B₁₂. In: Ziegler E, Filer LJ Jr, Eds. *Present Knowledge in Nutrition*, 7th ed. Washington, DC: International Life Sciences Institute Press, pp191–205, 1996.
 34. Watanabe F, Katsura H, Takenaka S, Enomoto T, Miyamoto E, Nakatsuka T, Nakano Y. Characterization of vitamin B₁₂ compounds from edible shellfish, clam, oyster, and mussel. *Int J Food Sci Nutr* 52: 263–268, 2001.
 35. Kimura N, Fukuwatari T, Sasaki R, Hayakawa F, Shibata K. Vitamin intake in Japanese women college students. *J Nutr Sci Vitaminol* 49: 149–155, 2003.
 36. Nishioka M, Kanosue F, Tanioka Y, Miyamoto E, Watanabe F. Characterization of vitamin B₁₂ in skipjack meats and loss of the vitamin from the fish meats by various cooking conditions. *Vitamins (Japanese)* 80:507–511, 2006.
 37. Nishioka M, Tanioka Y, Miyamoto E, Enomoto T, Watanabe F. TLC analysis of a corrinoid compound from dark muscle of the yellowfin tuna (*Thunnus albacares*). *J Liq Chrom Rel Technol* 30:1–8, 2007.
 38. Doscherholmen A, McMahon J, Economon P. Vitamin B₁₂ absorption from fish. *Proc Soc Exp Biol Med* 167:480–484, 1981.
 39. Report of the Subdivision on Resources. In: *Standard Tables of Food Composition in Japan*, 5th ed. The Council for Science and Technology, Ministry of Education, Culture, Sports, Science, and Technology, Japan, pp150–151, 2005.
 40. Adachi S, Miyamoto E, Watanabe F, Enomoto T, Kuda T, Hayashi M, Nakano Y. Purification and characterization of a corrinoid compound from a Japanese salted and fermented salmon kidney “Mefun.” *J Liq Chrom Rel Technol* 28:2561–2569, 2005.
 41. Areekul S, Boonyananta C, Matrakul D, Chantachum Y. Determination of vitamin B₁₂ in fish sauce in Thailand. *J Med Assoc Thailand* 55:243–248, 1972.
 42. Takenaka S, Enomoto T, Tsuyama S, Watanabe F. TLC analysis of corrinoid compounds in fish sauce. *J Liq Chrom Rel Technol* 26:2703–2707, 2003.
 43. Watanabe F, Michihata T, Takenaka S, Kittaka-Katsura H, Enomoto T, Miyamoto E, Adachi A. Purification and characterization of corrinoid compounds from a Japanese fish sauce. *J Liq Chrom Rel Technol* 27: 2113–2119, 2004.
 44. Miyamoto E, Kittaka-Katsura H, Adachi S, Watanabe F. Assay of vitamin B₁₂ in edible bamboo shoots. *Vitamins (Japanese)* 79:329–332, 2005.
 45. Mozafar A. Enrichment of some B-vitamins in plants with application of organic fertilizers. *Plant Soil* 167:305–311, 1994.
 46. Mozafar A, Oertli JJ. Uptake of a microbially-produced vitamin (B12) by soybean. *Plant Soil* 139:23–30, 1992.
 47. Sato K, Kudo, Y, Muramatsu K. Incorporation of a high level of vitamin B₁₂ into a vegetable, kaiware daikon (Japanese radish sprout), by the absorption from its seeds. *Biochim Biophys Acta* 1672:135–137, 2004.
 48. Kittaka-Katsura H, Watanabe F, Nakano Y. Occurrence of vitamin B₁₂ in green, blue, red, and black tea leaves. *J Nutr Sci Vitaminol* 50:438–440, 2004.
 49. Kittaka-Katsura H, Ebara S, Watanabe F, Nakano Y. Characterization of corrinoid compounds from a Japanese black tea (Batabata-cha) fermented by bacteria. *J Agric Food Chem* 52:909–911, 2004.
 50. Nout MJR, Rombouts FM. Recent developments in tempe research. *J Appl Bacteriol* 69:609–633, 1990.
 51. Denter J, Bisping, B. Formation of B-vitamins by bacteria during the soaking process of soybeans for tempe fermentation. *Int J Food Microbiol* 22:23–31, 1994.
 52. Okada N, Hadjoetomo PS, Nikkuni S, Katoh K, Ohta T. Vitamin B₁₂ content of fermented foods in the tropics. *Rept Nalt Food Res Inst* 43: 126–129, 1983.
 53. Dagnelie PC, van Staveren WA, van den Berg H. Vitamin B-12 from algae appears not to be bioavailable. *Am J Clin Nutr* 53:695–697, 1991.
 54. Watanabe F, Takenaka S, Katsura H, Miyamoto E, Abe K, Tamura Y, Nakatsuka T, Nakano Y. Characterization of a vitamin B₁₂ compound in the edible purple laver, *Porphyra yezoensis*. *Biosci Biotechnol Biochem* 64:2712–2715, 2000.
 55. Watanabe F, Katsura H, Miyamoto E, Takenaka S, Abe K, Yamasaki Y, Nakano Y. Characterization of vitamin B₁₂ in an edible green laver (*Enteromorpha prolifera*). *Appl Biol Sci* 5:99–107, 1999.
 56. Kittaka-Katsura H, Fujita T, Watanabe F, Nakano Y. Purification and characterization of a corrinoid-compound from *Chlorella* tablets as an algal health food. *J Agric Food Chem* 50:4994–4997, 2002.
 57. Miyamoto E, Watanabe F, Ebara S, Takenaka S, Takenaka H, Yamaguchi Y, Tanaka N, Inui H, Nakano Y. Characterization of a vitamin B₁₂ compound from unicellular coccolithophorid alga (*Pleurochrysis carterae*). *J Agric Food Chem* 49:3486–3489, 2001.
 58. Takenaka S, Sugiyama S, Ebara S, Miyamoto E, Abe K, Tamura Y, Watanabe F, Tsuyama S, Nakano Y. Feeding dried purple laver (nori) to vitamin B₁₂-deficient rats significantly improves vitamin B-12 status. *Brit J Nutr* 85:699–703, 2001.
 59. Suzuki H. Serum vitamin B₁₂ levels in young vegans who eat brown rice. *J Nutr Sci Vitaminol* 41:587–594, 1995.
 60. Rauma AL, Torronen R, Hanninen O, Mykkänen H. Vitamin B-12 status of long-term adherents of a strict uncooked vegan diet (“living food diet”) is compromised. *J Nutr* 125:2511–2515, 1995.
 61. Pulz O, Gross W. Valuable products from biotechnology of microalgae. *Appl Microbiol Biotechnol* 65:635–648, 2004.
 62. van den Berg H, Dagnelie PC, van Staveren WA. Vitamin B₁₂ and seaweed. *Lancet* 1:242–243, 1988.
 63. Watanabe F, Katsura H, Takenaka S, Fujita T, Abe K, Tamura Y, Nakatsuka T, Nakano Y. Pseudovitamin B₁₂ is the predominate cobamide of an algal health food, spirulina tablets. *J Agric Food Chem* 47:4736–4741, 1999.
 64. Stüpperich E, Nexø E. Effect of the cobalt-N coordination on the cobamide recognition by the human vitamin B₁₂ binding proteins intrinsic factor, transcobalamin, and haptocorrin. *Eur J Biochem* 199: 299–303, 1991.
 65. Brandt LJ, Goldberg L, Bernstein LH, Greenberg G. The effect of bacterially produced vitamin B-12 analogues (cobamides) on the in vitro absorption of cyanocobalamin. *Am J Clin Nutr* 32:1832–1836, 1979.
 66. Herbert V, Drivas G. Spirulina and vitamin B₁₂. *JAMA* 248:3096–3097, 1982.
 67. Herbert V. Vitamin B-12: plant sources, requirements, and assay. *Am J Clin Nutr* 48:852–858, 1988.
 68. van den Berg H, Brandsen L, Sinkeldam BJ. Vitamin B₁₂ content and bioavailability of spirulina and nori in rats. *J Nutr Biochem* 2:314–318, 1991.
 69. Kay RA. Microalgae as food and supplement. *Crit Rev Food Sci Nutr* 30:555–573, 1991.
 70. Miyamoto E, Tanioka Y, Nakao T, Barla F, Inui H, Fujita T, Watanabe F, Nakano Y. Purification and characterization of a corrinoid-compound in an edible cyanobacterium *Aphanizomenon flos-aquae* as a nutritional supplementary food. *J Agric Food Chem* 54:9604–9607, 2006.

71. Watanabe F, Miyamoto E, Fujita T, Tanioka Y, Nakano Y. Characterization of a corrinoid compound in the edible (blue-green) algae, *suizenji-nori*. *Biosci Biotechnol Biochem* 70:3066–3068, 2006.
72. Watanabe F, Tanioka Y, Miyamoto E, Fujita T, Takenaka H, Nakano Y. Purification and characterization of corrinoid-compounds from the dried powder of an edible cyanobacterium, *Nostoc commune* (Ishikurage). *J Nutr Sci Vitaminol* 53:183–186, 2007.
73. Tucker KL, Olson B, Bakun P, Dallal GE, Selhub J, Rosenberg IH. Breakfast cereal fortified with folic acid, vitamin B-6, and vitamin B-12 increases vitamin concentrations and reduces homocysteine concentrations: a randomized trial. *Am J Clin Nutr* 79:805–811, 2004.
74. Watanabe F. Vitamin B₁₂ from edible algae-from food science to molecular biology. *Vitamins (Japanese)* 81:49–55, 2007.