

14. Vitamin B₁₂

14.1 Role of vitamin B₁₂ in human metabolic processes

Although the nutritional literature still uses the term vitamin B₁₂, a more specific name for vitamin B₁₂ is cobalamin. Vitamin B₁₂ is the largest of the B complex vitamins, with a relative molecular mass of over 1000. It consists of a corrin ring made up of four pyrroles with cobalt at the centre of the ring (1, 2).

There are several vitamin B₁₂-dependent enzymes in bacteria and algae, but no species of plants have the enzymes necessary for vitamin B₁₂ synthesis. This fact has significant implications for the dietary sources and availability of vitamin B₁₂. In mammalian cells, there are only two vitamin B₁₂-dependent enzymes (3). One of these enzymes, methionine synthase, uses the chemical form of the vitamin which has a methyl group attached to the cobalt and is called methylcobalamin (see Chapter 15, Figure 15.2). The other enzyme, methylmalonyl coenzyme (CoA) mutase, uses a form of vitamin B₁₂ that has a 5'-deoxyadenosyl moiety attached to the cobalt and is called 5'-deoxyadenosylcobalamin, or coenzyme B₁₂. In nature, there are two other forms of vitamin B₁₂: hydroxycobalamin and aquacobalamin, where hydroxyl and water groups, respectively, are attached to the cobalt. The synthetic form of vitamin B₁₂ found in supplements and fortified foods is cyanocobalamin, which has cyanide attached to the cobalt. These three forms of vitamin B₁₂ are enzymatically activated to the methyl- or deoxyadenosylcobalamins in all mammalian cells.

14.2 Dietary sources and availability

Most microorganisms, including bacteria and algae, synthesize vitamin B₁₂, and they constitute the only source of the vitamin (4). The vitamin B₁₂ synthesized in microorganisms enters the human food chain through incorporation into food of animal origin. In many animals, gastrointestinal fermentation supports the growth of these vitamin B₁₂ synthesizing microorganisms, and subsequently the vitamin is absorbed and incorporated into the animal tissues. This is particularly true for the liver, where vitamin B₁₂ is stored in large con-

centrations. Products from herbivorous animals, such as milk, meat, and eggs, thus constitute important dietary sources of the vitamin, unless the animal is subsisting in one of the many regions known to be geochemically deficient in cobalt (5). Milk from cows and humans contains binders with very high affinity for vitamin B₁₂, though whether they hinder or promote intestinal absorption is not entirely clear. Omnivores and carnivores, including humans, derive dietary vitamin B₁₂ almost exclusively from animal tissues or products (i.e. milk, butter, cheese, eggs, meat, poultry). It appears that the vitamin B₁₂ required by humans is not derived from microflora in any appreciable quantities, although vegetable fermentation preparations have been reported as being possible sources of vitamin B₁₂ (6).

14.3 Absorption

The absorption of vitamin B₁₂ in humans is complex (1, 2). Vitamin B₁₂ in food is bound to proteins and is only released by the action of a high concentration of hydrochloric acid present in the stomach. This process results in the free form of the vitamin, which is immediately bound to a mixture of glycoproteins secreted by the stomach and salivary glands. These glycoproteins, called R-binders (or haptocorrins), protect vitamin B₁₂ from chemical denaturation in the stomach. The stomach's parietal cells, which secrete hydrochloric acid, also secrete a glycoprotein called intrinsic factor. Intrinsic factor binds vitamin B₁₂ and ultimately enables its active absorption. Although the formation of the vitamin B₁₂-intrinsic factor complex was initially thought to happen in the stomach, it is now clear that this is not the case. At an acidic pH, the affinity of the intrinsic factor for vitamin B₁₂ is low whereas its affinity for the R-binders is high. When the contents of the stomach enter the duodenum, the R-binders become partly digested by the pancreatic proteases, which in turn causes them to release their vitamin B₁₂. Because the pH in the duodenum is more neutral than that in the stomach, the intrinsic factor has a high binding affinity to vitamin B₁₂, and it quickly binds the vitamin as it is released from the R-binders. The vitamin B₁₂-intrinsic factor complex then proceeds to the lower end of the small intestine, where it is absorbed by phagocytosis by specific ileal receptors (1, 2).

14.4 Populations at risk for, and consequences of, vitamin B₁₂ deficiency

14.4.1 Vegetarians

Because plants do not synthesize vitamin B₁₂, individuals who consume diets completely free of animal products (vegan diets) are at risk of vitamin B₁₂ defi-

ciency. This is not true of lacto-ovo vegetarians, who consume the vitamin in eggs, milk, and other dairy products.

14.4.2 Pernicious anaemia

Malabsorption of vitamin B₁₂ can occur at several points during digestion (1, 4). By far the most important condition resulting in vitamin B₁₂ malabsorption is the autoimmune disease called pernicious anaemia (PA). In most cases of PA, antibodies are produced against the parietal cells causing them to atrophy, and lose their ability to produce intrinsic factor and secrete hydrochloric acid. In some forms of PA, the parietal cells remain intact but autoantibodies are produced against the intrinsic factor itself and attach to it, thus preventing it from binding vitamin B₁₂. In another less common form of PA, the antibodies allow vitamin B₁₂ to bind to the intrinsic factor but prevent the absorption of the intrinsic factor–vitamin B₁₂ complex by the ileal receptors. As is the case with most autoimmune diseases, the incidence of PA increases markedly with age. In most ethnic groups, it is virtually unknown to occur before the age of 50, with a progressive rise in incidence thereafter (4). However, African American populations are known to have an earlier age of presentation (4). In addition to causing malabsorption of dietary vitamin B₁₂, PA also results in an inability to reabsorb the vitamin B₁₂ which is secreted in the bile. Biliary secretion of vitamin B₁₂ is estimated to be between 0.3 and 0.5 µg/day. Interruption of this so-called enterohepatic circulation of vitamin B₁₂ causes the body to go into a significant negative balance for the vitamin. Although the body typically has sufficient vitamin B₁₂ stores to last 3–5 years, once PA has been established, the lack of absorption of new vitamin B₁₂ is compounded by the loss of the vitamin because of negative balance. When the stores have been depleted, the final stages of deficiency are often quite rapid, resulting in death in a period of months if left untreated.

14.4.3 Atrophic gastritis

Historically, PA was considered to be the major cause of vitamin B₁₂ deficiency, but it was a fairly rare condition, perhaps affecting between one and a few per cent of elderly populations. More recently, it has been suggested that a far more common problem is that of hypochlorhydria associated with atrophic gastritis, where there is a progressive reduction with age of the ability of the parietal cells to secrete hydrochloric acid (7). It is claimed that perhaps up to one quarter of elderly subjects could have various degrees of hypochlorhydria as a result of atrophic gastritis. It has also been suggested that bacterial overgrowth in the stomach and intestine in individuals suffering from atrophic gastritis may also reduce vitamin B₁₂ absorption. The

absence of acid in the stomach is postulated to prevent the release of protein-bound vitamin B₁₂ contained in food but not to interfere with the absorption of the free vitamin B₁₂ found in fortified foods or supplements. Atrophic gastritis does not prevent the reabsorption of biliary vitamin B₁₂ and therefore does not result in the negative balance seen in individuals with PA. Nonetheless, it is agreed that with time, a reduction in the amount of vitamin B₁₂ absorbed from the diet will eventually deplete vitamin B₁₂ stores, resulting in overt deficiency.

When considering recommended nutrient intakes (RNIs) for vitamin B₁₂ for the elderly, it is important to take into account the absorption of vitamin B₁₂ from sources such as fortified foods or supplements as compared with dietary vitamin B₁₂. In the latter instances, it is clear that absorption of intakes of less than 1.5–2.0 µg/day is complete—that is, for daily intakes of less than 1.5–2.0 µg of free vitamin B₁₂, the intrinsic factor-mediated system absorbs that entire amount. It is probable that this is also true of vitamin B₁₂ in fortified foods, although this has not been specifically examined. However, absorption of food-bound vitamin B₁₂ has been reported to vary from 9% to 60% depending on the study and the source of the vitamin, which is perhaps related to its incomplete release from food (8). This has led many to estimate absorption as being up to 50% to correct for the bioavailability of vitamin B₁₂ from food.

14.5 Vitamin B₁₂ interaction with folate or folic acid

One of the vitamin B₁₂-dependent enzymes, methionine synthase, functions in one of the two folate cycles, namely, the methylation cycle (see Chapter 15). This cycle is necessary to maintain availability of the methyl donor, *S*-adenosylmethionine. Interruption of the cycle reduces the level of *S*-adenosylmethionine. This occurs in PA and other causes of vitamin B₁₂ deficiency, producing as a result demyelination of the peripheral nerves and the spinal column, giving rise to the clinical condition called subacute combined degeneration (1, 2). This neuropathy is one of the main presenting conditions in PA. The other principal presenting condition in PA is a megaloblastic anaemia morphologically identical to that seen in folate deficiency. Disruption of the methylation cycle also causes a lack of DNA biosynthesis and anaemia.

The methyl trap hypothesis is based on the fact that once the cofactor 5,10-methylenetetrahydrofolate is reduced by its reductase to form 5-methyltetrahydrofolate, the reverse reaction cannot occur. This suggests that the only way for the 5-methyltetrahydrofolate to be recycled to tetrahydrofolate, and thus to participate in DNA biosynthesis and cell division, is through the vitamin B₁₂-dependent enzyme methionine synthase. When the activity of this

synthase is compromised, as it would be in PA, the cellular folate will become progressively trapped as 5-methyltetrahydrofolate (see Chapter 15, Figure 15.2). This will result in a cellular pseudo-folate deficiency where, despite adequate amounts of folate, anaemia will develop, which is identical to that seen in true folate deficiency. Clinical symptoms of PA, therefore, include neuropathy, anaemia, or both. Treatment with vitamin B₁₂, if given intramuscularly, will reactivate methionine synthase, allowing myelination to restart. The trapped folate will be released and DNA synthesis and generation of red cells will cure the anaemia. Treatment with high concentrations of folic acid will treat the anaemia but not the neuropathy of PA. It should be stressed that the so-called “masking” of the anaemia of PA is generally agreed not to occur at concentrations of folate found in food or at intakes of the synthetic form of folic acid at usual RNI levels of 200 or 400 µg/day (1). However, there is some evidence that amounts less than 400 µg may cause a haematologic response and thus potentially treat the anaemia (9). The masking of the anaemia definitely occurs at high concentrations of folic acid (>1000 µg/day). This becomes a concern when considering fortification with synthetic folic acid of a dietary staple such as flour (see Chapter 15).

In humans, the vitamin B₁₂-dependent enzyme methylmalonyl CoA mutase functions both in the metabolism of propionate and certain amino acids—converting them into succinyl CoA—and in the subsequent metabolism of these amino acids via the citric acid cycle. It is clear that in vitamin B₁₂ deficiency the activity of the mutase is compromised, resulting in high plasma or urine concentrations of methylmalonic acid (MMA), a degradation product of methylmalonyl CoA mutase. In adults, this mutase does not appear to have any vital function, but it clearly has an important role during embryonic life and in early development. Children deficient in this enzyme, through rare genetic mutations, suffer from mental retardation and other developmental defects.

14.6 Criteria for assessing vitamin B₁₂ status

Traditionally it was thought that low vitamin B₁₂ status was accompanied by a low serum or plasma vitamin B₁₂ level (4). Recently, Lindenbaum et al. (10) challenged this assumption, by suggesting that a proportion of people with normal serum and plasma vitamin B₁₂ levels are in fact vitamin B₁₂ deficient. They also suggested that elevation of plasma homocysteine and plasma MMA are more sensitive indicators of vitamin B₁₂ status. Although plasma homocysteine can also be elevated because of folate or vitamin B₆ deficiency, elevation of MMA apparently always occurs with poor vitamin B₁₂ status. However, there may be other reasons why MMA is elevated, such as renal

insufficiency, so the elevation of MMA, in itself, is not diagnostic. Thus, low serum or plasma levels of vitamin B₁₂ should be the first indication of poor status and this could be confirmed by an elevated MMA if this assay was available.

14.7 Recommendations for vitamin B₁₂ intakes

The Food and Nutrition Board of the National Academy of Sciences (NAS) Institute of Medicine (8) has recently conducted an exhaustive review of the evidence regarding vitamin B₁₂ intake, status, and health implications for all age groups, including the periods of pregnancy and lactation. This review has led to calculations of what they have called an estimated average requirement (EAR), which is defined by NAS as “the daily intake value that is estimated to meet the requirement, as defined by the specific indicator of adequacy, in half of the individuals in a life-stage or gender group” (8). The NAS then estimated a recommended dietary allowance (RDA) for vitamin B₁₂, as this daily intake value plus 2 standard deviations (SDs).

Some members of the present FAO/WHO Consultation were involved in the preparation and review of the NAS recommendations and judge them to be the best estimates currently available. The FAO/WHO Consultation thus felt it appropriate to adopt the same approach used by the NAS in deriving the RNIs for vitamin B₁₂. Therefore, the EARs given in Table 14.1 are the same as those proposed by the NAS, and the RNIs (which are equivalent to

TABLE 14.1
Estimated average requirements (EARs) and recommended nutrient intakes (RNIs) for vitamin B₁₂, by group

Group	EAR (µg/day)	RNI (µg/day)
<i>Infants and children</i>		
0–6 months	0.3	0.4
7–12 months	0.6	0.7
1–3 years	0.7	0.9
4–6 years	1.0	1.2
7–9 years	1.5	1.8
<i>Adolescents</i>		
10–18 years	2.0	2.4
<i>Adults</i>		
19–65 years	2.0	2.4
65+ years	2.0	2.4
<i>Pregnant women</i>	2.2	2.6
<i>Lactating women</i>	2.4	2.8

Source: adapted from reference (8).

the RDAs used by the NAS) calculated as the EAR plus 2 SD. Supporting evidence for the recommendations for each age group is summarized below.

14.7.1 Infants

As with other nutrients, the principal way to determine requirements of infants is to examine the levels in milk from mothers on adequate diets. There is a wide difference in the vitamin B₁₂ values reported in human milk because of differences in methodology. The previous FAO/WHO Expert Consultation (11) based their recommendations on milk vitamin B₁₂ values of normal women of about 0.4 µg/l. For an average milk production of 0.75 l/day, the vitamin B₁₂ intake by infants would be 0.3 µg/day (12). Other studies have reported concentrations of vitamin B₁₂ in human milk in the range 0.4–0.8 µg/l (13–17). Although daily intakes ranging from 0.02 to 0.05 µg/day have been found to prevent deficiency (18, 19), these intakes are totally inadequate for long-term health. Thus, based on the assumption that human milk contains enough vitamin B₁₂ for optimum health, an EAR between 0.3 and 0.6 µg/day seems reasonable giving an RNI of between 0.4 and 0.7 µg/day. It would seem appropriate to use the lower RNI figure of 0.4 µg/day for infants aged 0–6 months and the higher RNI figure of 0.7 µg/day for infants aged 7–12 months (Table 14.1).

14.7.2 Children

The Food and Nutrition Board of the NAS Institute of Medicine (8) suggested the same intakes for adolescents as those for adults (see section 14.7.3) with progressive reduction of intake for younger groups.

14.7.3 Adults

Several lines of evidence point to an adult average requirement of about 2.0 µg/day. The amount of intramuscular vitamin B₁₂ needed to maintain remission in people with PA suggests a requirement of about 1.5 µg/day (10), but they would also be losing 0.3–0.5 µg/day through interruption of their enterohepatic circulation. This might suggest a requirement of 0.7–1.0 µg/day for those without PA. Because vitamin B₁₂ is not completely absorbed from food, an adjustment of 50% has to be added, giving a range of 1.4–2.0 µg/day (4). Therapeutic response to dietary vitamin B₁₂ suggests a minimum requirement of something less than 1.0 µg/day (8), which again suggests a requirement of 2.0 µg/day, allowing for the conservative correction that only half of dietary vitamin B₁₂ is absorbed (8). Diets containing 1.8 µg/day seemed to maintain adequate status but intakes lower than this resulted in subjects showing some signs of deficiency (8). Furthermore, dietary intakes

of less than 1.5 µg/day were reported to be inadequate in some subjects (20).

In summary, the average requirement could be said to be 2 µg/day (8). Assuming the variability of the requirements for vitamin B₁₂ is accounted for by adding 2 SDs, the RNI for adults and the elderly becomes 2.4 µg/day.

14.7.4 Pregnant women

The previous FAO/WHO Expert Consultation (11) estimated that 0.1–0.2 µg/day of vitamin B₁₂ is transferred to the fetus during the last two trimesters of pregnancy. On the basis of fetal liver content from postmortem samples (21–23), there is further evidence that the fetus accumulates, on average, 0.1–0.2 µg/day of vitamin B₁₂ during pregnancies of women with diets which provide adequate levels of vitamin B₁₂. It has been reported that children born to vegetarians or other women with a low vitamin B₁₂ intake subsequently develop signs of clinical vitamin B₁₂ deficiency such as neuropathy (13). Therefore, in order to derive an EAR for pregnant women, 0.2 µg/day of vitamin B₁₂ was added to the EAR for adults, to give an EAR of 2.2 µg/day and a RNI of 2.6 µg/day during pregnancy.

14.7.5 Lactating women

It is estimated that 0.4 µg/day of vitamin B₁₂ is found in the human milk of women with adequate vitamin B₁₂ status (8). Therefore, an extra 0.4 µg/day of vitamin B₁₂ is needed during lactation in addition to the normal adult requirement of 2.0 µg/day, giving a total EAR of 2.4 µg/day and a RNI of 2.8 µg/day during lactation.

14.8 Upper limits

The absorption of vitamin B₁₂ mediated by the glycoprotein, intrinsic factor, is limited to 1.5–2.0 µg per meal because of the limited capacity of the receptors. In addition, between 1% and 3% of any particular oral administration of vitamin B₁₂ is absorbed by passive diffusion. Thus, if 1000 µg vitamin B₁₂ (sometimes used to treat those with PA) is taken orally, the amount absorbed would be 2.0 µg by active absorption plus up to about 30 µg by passive diffusion. Intake of 1000 µg vitamin B₁₂ has never been reported to have any side-effects (8). Similar large amounts have been used in some preparations of nutritional supplements without apparent ill effects. However, there are no established benefits for such amounts. Such high intakes thus represent no benefit in those without malabsorption and should probably be avoided.

14.9 Recommendations for future research

Because they do not consume any animal products, vegans are at risk of vitamin B₁₂ deficiency. It is generally agreed that in some communities the only source of vitamin B₁₂ is from contamination of food by microorganisms. When vegans move to countries where standards of hygiene are more stringent, there is good evidence that risk of vitamin B₁₂ deficiency increases in adults and, particularly, in children born to and breastfed by women who are strict vegans.

As standards of hygiene improve in developing countries, there is a concern that the prevalence of vitamin B₁₂ deficiency might increase. This should be ascertained by estimating plasma vitamin B₁₂ levels, preferably in conjunction with plasma MMA levels in representative adult populations and in infants.

Further research needs include the following:

- ascertaining the contribution that fermented vegetable foods make to the vitamin B₁₂ status of vegan communities;
- investigating the prevalence of atrophic gastritis in developing countries to determine its extent in exacerbating vitamin B₁₂ deficiency.

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