Chapter 5
Vitamin B_{12}

Role of vitamin B_{12} in human metabolic processes

Although the nutritional literature still uses the term vitamin B_{12}, a more specific name for vitamin B_{12} is cobalamin. Vitamin B_{12} is the largest of the B complex vitamins, with a molecular weight of over 1000. It consists of a corrin ring made up of four pyrroles with cobalt at the center of the ring (*1, 2*).

There are several vitamin B_{12}–dependent enzymes in bacteria and algae, but no species of plants have the enzymes necessary for vitamin B_{12} synthesis. This fact has significant implications for the dietary sources and availability of vitamin B_{12}. In mammalian cells there are only two vitamin B_{12}–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 ***Figure 7*** in Chapter 4.). The other enzyme, methylmalonyl CoA mutase, uses vitamin B_{12} with a 5'-adeoxyadenosyl moiety attached to the cobalt and is called 5'-deoxyaldenosylcobalamin, or coenzyme B_{12}. In nature there are two other forms of vitamin B_{12}: hydroxycobalamin and aquacobalamin, where hydroxyl and water groups, respectively, are attached to the cobalt. The synthetic form of vitamin B_{12} found in supplements and fortified foods is cyanocobalamin, which has cyanide attached to the cobalt. These three forms of B_{12} are enzymatically activated to the methyl- or deoxyadenosylcobalamins in all mammalian cells.

Dietary sources and availability

Most microorganisms, including bacteria and algae, synthesise vitamin B_{12}, and they constitute the only source of the vitamin (*4*). The vitamin B_{12} synthesised 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 B_{12}–synthesising microorganisms, and subsequently the vitamin is absorbed and incorporated into the animal tissues. This is particularly true for the liver, where vitamin B_{12} is stored in large concentrations. Products from these herbivorous animals, such as milk, meat, and eggs, 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 B_{12}, whether they hinder or promote intestinal absorption is not entirely clear. Omnivores and carnivores, including humans, derive dietary vitamin B_{12} from animal tissues or products (i.e., milk, butter, cheese, eggs, meat, poultry, etc.). It appears that no significant amount of the required vitamin B_{12} by humans is derived from microflora, although vegetable fermentation preparations have also been reported as being possible sources of vitamin B_{12} (6).

Absorption

The absorption of vitamin B_{12} in humans is complex (*1, 2*). Vitamin B_{12} in food is bound to proteins and is released from the proteins 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 B_{12}

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 B_{12} and ultimately enables its active absorption. Although the formation of the vitamin B_{12} – 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 B_{12} 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 causes them to release their vitamin B_{12}. Because the pH in the duodenum is more neutral than that in the stomach, the intrinsic factor has a high binding affinity to vitamin B_{12}, and it quickly binds the vitamin as it is released from the R-binders. The vitamin B_{12}–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*).

Populations at risk for and consequences of vitamin B_{12} deficiency

Vegetarians

Because plants do not synthesise vitamin B_{12}, individuals who consume diets completely free of animal products (vegan diets) are at risk of vitamin B_{12} deficiency. This is not true of lacto-ovo-vegetarians, who consume the vitamin in eggs, milk, and other dairy products.

Pernicious anaemia

Malabsorption of vitamin B_{12} can occur at several points during digestion (*1, 4*). By far the most important condition resulting in vitamin B_{12} malabsorption is the auto-immune disease called pernicious anaemia (PA). In most cases of PA, antibodies are produced against the parietal cells causing them to atrophy, lose their ability to produce intrinsic factor, and secrete hydrochloric acid. In some forms of PA the parietal cells remain intact but auto-antiobodies are produced against the intrinsic factor itself and attach to it, thus preventing it from binding vitamin B_{12}. In another less common form of PA, the antibodies allow vitamin B_{12} to bind to the intrinsic factor but prevent the absorption of the intrinsic factor–vitamin B_{12} complex by the ileal receptors. As is the case with most auto-immune 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 B_{12}, PA also results in an inability to reabsorb the vitamin B_{12} which is secreted in the bile. Biliary secretion of vitamin B_{12} is estimated to be between 0.3 and 0.5 µg/day. Interruption of this so-called enterohepatic circulation of vitamin B_{12} causes the body to go into a significant negative balance for the vitamin. Although the body typically has sufficient vitamin B_{12} stores to last 3–5 years, once PA has been established the lack of absorption of new vitamin B_{12} 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.

Atrophic gastritis

Historically, PA was considered to be the major cause of vitamin B_{12} deficiency, but it was a fairly rare condition, perhaps affecting 1 percent to a few percent 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 B_{12}

absorption. This absence of acid is postulated to prevent the release of protein-bound vitamin B_{12} contained in food but not to interfere with the absorption of the free vitamin B_{12} found in fortified foods or supplements. Atrophic gastritis does not prevent the reabsorption of bilary vitamin B_{12} and therefore does not result in the negative balance seen in individuals with PA. However, it is agreed that with time, a reduction in the amount of vitamin B_{12} absorbed from the diet will eventually deplete even the usually adequate vitamin B_{12} stores, resulting in overt deficiency.

When considering recommended nutrient intakes (RNIs) for vitamin B_{12} for the elderly, it is important to take into account the absorption of vitamin B_{12} from sources such as fortified foods or supplements as compared with dietary vitamin B_{12}. 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 intakes of less than 1.5–2.0 µg of free vitamin B_{12}, the intrinsic factor – mediated system absorbs all of that amount. It is probable that this is also true of vitamin B_{12} in fortified foods, although this has not specifically been examined. However, absorption of food-bound vitamin B_{12} has been reported to vary from 9 percent to 60 percent 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 percent to correct for bio-availability of absorption from food.

Vitamin B_{12} interaction with folate or folic acid

One of the vitamin B_{12} – dependent enzymes, methionine synthase, functions in one of the two folate cycles (see ***Chapter 4***) – the methylation cycle. This cycle is necessary to maintain availability of the methyl donor *S*-adenosylmethionine; interruption reduces the wide range of methylated products. One such important methylation is that of myelin basic protein. Reductions in the level of *S*-adenosylmethionine seen in PA and other causes of vitamin B_{12} deficiency produce demyelination of the peripheral nerves and the spinal column, called sub-acute 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 should cause 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 methyltetrahydrofolate to be recycled to tetrahydrofolate, and thus to participate in DNA biosynthesis and cell division, is through the vitamin B_{12} – 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. This will result in a cellular pseudo folate deficiency where despite adequate amounts of folate an anaemia will develop that is identical to that seen in true folate deficiency. Clinical symptoms of PA, therefore, include neuropathy, anaemia, or both. Treatment with vitamin B_{12}, 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 found 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 4***).

In humans the vitamin B_{12} – dependent enzyme methylmalonyl coenzyme A (CoA) mutase functions in the metabolism of propionate and certain of the amino acids, converting them into succinyl CoA, and in their subsequent metabolism via the citric acid cycle. It is clear that in vitamin B_{12} 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. 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.

Assessment of vitamin B_{12} status

Traditionally it was thought that low vitamin B_{12} status was accompanied by a low serum or plasma vitamin B_{12} level (*4*). Recently this has been challenged by Lindenbaum *et al*.(*10*), who suggested that a proportion of people with normal vitamin B_{12} levels are in fact vitamin B_{12} deficient. They also suggested that elevation of plasma homo-cysteine and plasma MMA are more sensitive indicators of vitamin B_{12} status. Although plasma homo-cysteine may also be elevated because of folate or vitamin B_6 deficiency, elevation of MMA apparently always occurs with poor vitamin B_{12} status. There may be other reasons why MMA is elevated, such as renal insufficiency, so the elevation of itself is not diagnostic. Many would feel that low or decreased plasma vitamin B_{12} levels should be the first indication of poor status and that this could be confirmed by an elevated MMA if this assay was available.

Evidence on which to base a recommended intake

Recommendations for nutrient intake

The Food and Nutrition Board of the National Academy of Sciences (NAS) Institute of Medicine (*8*) has recently exhaustively reviewed the evidence of intake, status, and health for all age groups and during pregnancy and lactation. This review has lead to calculations of what they have called an estimated average requirement (EAR). The EAR 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)*. They have estimated the recommended dietary allowances to be this figure plus 2 standard deviations (SDs). Some members of the Food and Agriculture Organization of the United Nations and World Health Organization (FAO/WHO) Expert Consultation were involved in the preparation and review of the NAS recommendations and judge them to have been the best estimates based on available scientific literature. The FAO/WHO expert group felt it appropriate to adopt the same approach used by the NAS in deriving the RNIs. Therefore the RNIs suggested in ***Table 14*** are based on the NAS EARs plus 2 SDs.

Adults

Several lines of evidence point to an adult average requirement of about 2.0 µg/day. The amount of intramuscular vitamin B_{12} needed to maintain remission in people with PA suggests a requirement of about 1.5 µg/day (*10*), but they would also be losing 0.3–0.5µg/day through interruption of their enterohepatic circulation, which is not typical. This might suggest a requirement of 0.7–1.0 µg/day. Because vitamin B_{12} is not completely absorbed from food, an adjustment of 50 percent has to be added giving a range of 1.4–2.0 µg/day (*4*). Therapeutic response to ingested food vitamin B_{12} suggests a minimum requirement of something less than 1.0 µg/day (*8*). Diets containing 1.8 µg/day seemed to maintain adequate status but lower intakes showed some signs of deficiency. (*8*). Dietary intakes of less than 1.5 µg/day were reported to be inadequate in some subjects (*11*).

In summary, the average requirement could be said to be 2 µg/day (*8*). The variability of the requirements for vitamin B_{12} is accounted for by adding two SDs, that is, 2.4 µg/day as an RNI for adults, including the elderly.

Table 14

Estimated average requirement (EAR) and recommended nutrient intake (RNI) for vitamin B_{12}, by age group

Adapted from the US National Academy of Sciences (8).

Group	EAR µg/day	RNI µg/day
Infants and children		
0–6 months	0.32	0.4
7–12 months	0.32	0.5
1–3 years	0.7	0.9
4–6 years	1.0	1.2
7–9 years	1.5	1.8
Adolescents, 10–18 years	2.0	2.4
Adults		
19–65 years	2.0	2.4
65+ years	2.0	2.4
Pregnancy	2.2	2.6
Lactation	2.4	2.8

Children

The Food and Nutrition Board of the NAS Institute of Medicine (*8*) suggested the same intakes for adolescents with progressive reduction of intake for younger groups.

Pregnancy

The previous FAO/WHO (12) Expert Consultation suggested that 0.1–0.2 µg/day of vitamin B_{12} is transferred to the foetus (*13*) during the last two trimesters of pregnancy. On the basis of on foetal liver content from post-mortem samples (*14*, *15, 16*), there is further evidence that the foetus accumulates on average 0.1–0.2 µg/day during pregnancies of women with diets which have adequate vitamin B_{12}. It has been reported that children born to vegetarians or other women with a low vitamin B_{12} intake subsequently develop signs of clinical vitamin B_{12} deficiency such as neuropathy (*17*). Therefore, when calculating the EAR for pregnant women, 0.2 µg/day of vitamin B_{12} is added to the EAR for adults to result in an EAR of 2.2 µg/day and an RNI of 2.6 µg /day during pregnancy.

Lactation

It is estimated that 0.4 µg/day of vitamin B_{12} is found in the human milk of women with adequate vitamin B_{12} status (*8)*. Therefore an extra 0.4 µg/day of vitamin B_{12} 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 during lactation and an RNI of 2.8 µg/day.

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

B_{12} values reported in human milk because of differences in methodology. The previous FAO/WHO report (12) used milk vitamin B_{12} values of normal women of about 0.4 µg/l. For an average milk production of 0.75 l/day, the vitamin B_{12} intake by infants would be 0.3 µg/day (*18)*. Other studies have reported ranges of vitamin B_{12} in human milk to be 0.4–0.8 µg/L (*17, 19, 20, 21, 22*). Although daily intakes ranging from 0.02 to 0.05 µg/day have been found to prevent deficiency (*23, 24*), these intakes are totally inadequate for long-term health. An EAR of 0.3–0.6 µg/day would result in an RNI of 0.36–0.72 µg/d. It might be prudent to use the lower figure of 0.4 µg/day for the first 6 months of pregnancy and 0.7 µg/day for last trimester.

Upper limits

The absorption of vitamin B_{12} mediated by intrinsic factor is limited to 1.5–2.0 µg per meal because of the limited capacity of the receptors. In addition, between 1 percent and 3 percent of any particular oral administration of vitamin B_{12} is absorbed by passive diffusion. Thus, if 1000 µg vitamin B_{12} (sometimes used to treat those with PA) is taken orally, the amount absorbed would be 2.0 µg by active absorption plus about 30 µg by passive diffusion. This amount 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.

Future research

Because they do not consume any animal products, vegans are at risk of vitamin B_{12} deficiency. It is generally agreed that in some communities the only source of vitamin B_{12} 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 B_{12} deficiency increases in adults and, particularly, in children born to and breast-fed by women who are strict vegans.

- As standards of hygiene improve in developing countries, there is a concern that the prevalence of vitamin B_{12} deficiency might increase. This should be ascertained by estimating plasma vitamin B_{12} levels, preferably in conjunction with plasma MMA levels in representative adult populations and in infants.
- The contribution which fermented vegetable foods make to vitamin B_{12} status of vegan communities should be investigated.
- The prevalence of atrophic gastritis should be investigated in developing countries.

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