Understanding Vitamin B_{12}

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ANALYTIC REVIEW

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Abstract: *Vitamin B_{12} (B_{12}) is essential in activating folate needed in DNA synthesis. Inadequate intake results in the impairment of nerve transmission and inadequate synthesis of erythrocytes and other hematological cells. Two separate mechanisms of B_{12} absorption exist, a receptor-mediated endocytosis that occurs in the distal ileum and the mass-action pharmacologic mechanism. The recommended dietary allowance for B_{12} for adults issued by the Institute of Medicine is 2.4 μg/d. B_{12} is only found in meats and other foods of animal origin. B_{12} deficiency is widespread. Two main causes of deficiency include inadequate absorption and intake. Elderly and vegetarians are at highest risk for deficiency. Prevalence of deficiency ranges from 7% of the US population 3 years and older to 90% among vegans. The best way to assess deficiency is by using methylmalonic acid. Populations at risk could benefit from using B_{12} supplements and from fortification of flour.*

Keywords: cobalamin; B12; assessment; deficiency; food sources

Introduction

Vitamin B_{12} (B_{12}) was discovered in 1948 by Smith as an antipernicious factor.[1] B_{12} has the largest and most complex chemical structure of all vitamins.[2] It has been named cobalamin because it contains the rare element cobalt in its chemical structure. Cobalamin belongs to a group of cobalt-containing compounds known as corrinoids that contain a specific corrin ring. Unlike B_{12} analogues that are inactive in humans, the active forms of B_{12} have 3 distinct molecules: aminopropanol, sugar (eg, ribose), and a nucleotide attached to the corrin nuclei.[3] Methylcobalamin and adenosylcobalamin are 2 biologically active forms of B_{12}. Other forms such as hydroxocobalamin (or aquacobalamin) and cyanocobalamin must be metabolized to either of the 2 active forms in order to be used in human cells. A specific R-group attached to the cobalt element is what differentiates these forms of B_{12} chemically.

Function

B_{12} (methylcobalamin) is a coenzyme involved in the transfer of a methyl group in a methionine synthase-requiring reaction that converts homocysteine to methionine. B_{12} (adenosylcobalamin) is also essential in converting L-methylmalonyl-coenzyme A (CoA) to succinyl-CoA, a result of an action of another enzyme methylmalonyl-CoA mutase.[4] In the first reaction, B12 activates folate, which is essential in DNA synthesis and thus affects nucleic acid (DNA) synthesis. Since methionine is needed for the synthesis of myelin, a coating of the nerve pathways, B_{12} deficiency may result in inadequate myelin synthesis, maintenance, and repair and may impair nerve transmission. Methylmalonyl-CoA mutase is especially active in mitochondria, the energy production unit of cells. Other functions of B_{12} include erythropoiesis and synthesis of other hematological cells.

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Digestion and Absorption

B_{12} is only synthesized by microorganisms. B_{12} is not found in foods of plant origin.[3] It is, however, found in meats

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and foods of animal origin, including dairy products and eggs. In foods, B_{12} is bound to proteins known as R-proteins or R-binders. For B_{12} to be absorbed, the peptide bond that binds B_{12} to the protein carrier has to be broken down. The process of digestion and absorption takes place in a few stages and requires adequate synthesis of hydrochloric acid, proteases and an intrinsic factor (IF), a glycoprotein secreted by the parietal cells of the stomach. B_{12} is released from the R-binders found in foods by the actions of pepsin, an enzyme secreted in the stomach. This enzyme is secreted as pepsinogen and is activated by hydrochloric acid. Once released, B_{12} binds with R-binders (also known as haptocorrins) secreted with the saliva. Bound to these R-binders B_{12} travels into the small intestine wherein pancreatic proteases digest the R-binders, enabling B_{12} to be taken up by IF, forming IF-B12 (or IF-Cbl) complex.[3]

There are 2 separate mechanisms of B12 absorption into the blood stream. The first is a receptor-mediated endocytosis that occurs in the distal ileum. IF-Cbl receptor is only expressed in the distal ileum of the intestines. Once inside the enterocytes B_{12} is released from the IF and it then binds to holo-transcobalamin II protein (TCII), a protein carrier synthesized in the microvascular endothilium of the ileal villi.[5,6] Cell membranes internalize the TCII-Cbl complex by a process known as endocytosis.

The second mechanism of B_{12} absorption has been referred to as the mass-action pharmacologic mechanism.[3] In the presence of large amounts of B_{12}, such as in the case of ingesting B_{12} supplements, about 1% or less of free vitamin B_{12} is absorbed by diffusion across the epithelial ileum. This process does not require IF and bypasses IF-Cbl receptors.

Recommendation

The recommended dietary allowance (RDA) for B_{12} for adults issued by the Institute of Medicine is 2.4 µg/d.[7] The daily amount that is actually needed to maintain adequate serum level to promote erythropoiesis and other hematological functions is considerably smaller but this recommendation assumes a 50% absorption rate of B_{12} from the amount ingested with foods. The RDA issued by the Institute of Medicine is consistent with recommendations of other organizations. For example, the World Health Organization recommends an intake of 1.9 µg/d, the European Union, 3 µg/d, and the National Research Council, 2 µg/d.[7-9] Intake of relatively high doses of B_{12} does not appear to be associated with any detrimental health problems. Thus, the Institute of Medicine states that there is no sufficient scientific evidence to set a tolerable upper intake level for B_{12}.[7]

Food Sources and Bioavailability of B12

B_{12} in foods comes in several forms, including adenosylcobalamin, hydroxocobalamin, methylcobalamin, cyanocobalamin, and sulfitocobalamin.[2,3] Adenosylcobalamin and hydroxocobalamin are the predominant sources in foods. Hydroxocobalamin and methylcobalamin are better absorbed than the other forms.[10] Although B_{12} is found naturally only in meats and foods of animal origin, some foods believed to be made exclusively from plant foods (eg, cereal, breads, pies, and even cookies) do contain very small amounts of B_{12} because of either contamination during processing, adding of small amounts of ingredients derived from products of animal origin such as milk solids, or fortification.[11] However, the amount of B_{12} in the majority of these foods, except foods fortified with this vitamin, is negligible.

The highest amounts of B_{12} are found in organ meats such as the liver and in clams. The content of B_{12} in the liver varies from about 3.3 µg in whole chicken liver to more than 70 µg in 3 oz. of beef liver. Most other meats contain between 1 and 3 µg of B_{12} per 3 oz. Bioavailability of B_{12} from meats ranges from 10% from liver to as much as 90% from ground-cooked mutton patties.[2] Bioavailability depends on the quantity of meats ingested. B_{12} content of fish varies from 3.0 to 8.9 µg/100 g (about 3.5 oz). One of the best fish sources of B_{12} appears to be the dark muscle of skipjack (159 µg/100 g).[2]

Milk contains between 0.3 and 0.4 µg B_{12}/100 g. The absorption rate of B_{12} from milk is about 65%. B_{12} can be destroyed by heat. Boiling milk can destroy 30% to 50% of B_{12} depending on the duration of cooking and pasteurization destroys 5% to 10% of B_{12}. The B_{12} content of dairy products such as cheese or cottage cheese ranges between 20% and 60% of that of milk.[2]

B_{12} content of whole egg is between 0.9 and 1.4 µg/100 g.[2] Most of the B_{12} in an egg is found in the yolk. Bioavailability of B_{12} from eggs depends on the preparation method (eg, scrambled egg, boiled egg) ranging from less than 4% to a little more than 9%.[2,11,12]

Supplements and Fortified Foods

B_{12} is widely available as supplements in pharmacies and health food stores alike in the United States, mostly as cyanocobalamin. Other forms of this vitamin such as methylcobalamin and hydroxocobalamin can also be found. The dose of cyanocobalamin in supplements ranges from 100 to 5000 µg.

In the United States, many foods are fortified with B_{12}. This includes cereals such as Kellogg's Special K, Wheat Bran Flakes, Total, Total Raisin Bran, All Bran Original, soymilk, and soy meat analogues such as MorningstarFarms Burger Crumbles. These foods contain from less than the RDA to more than 200% of the RDA for B_{12}.[3] Additionally, 1 tablespoon of nutritional yeast contains 100% or more of the RDA of B_{12}.[13]

Bacteria-Synthesized B_{12} in the Small Intestine

Although a team of researchers led by Albert[14] found that lactobacilli, streptococci, bacteroides, and other enteral bacteria made some B_{12}, this amount does not seem to be adequate to maintain required B_{12} status in humans. In addition, these researchers used microbiologic assays in assessment of the B_{12} synthesis and this method measures both the active B_{12} and inactive B_{12} analogues.[3]

Thus, it is impossible to know how much of the isolated corrinoids was the active cobalamin.

Algae as a Source of B_{12}

Although in the past algae such as spirulina, nori, or kombu were believed to contain B_{12}, Herbert[3] showed not only that they almost exclusively contain inactive analogues of B_{12} but also that they may interfere with absorption and metabolism of the active B_{12} forms and thus, may contribute to the development of B_{12} deficiency.

Assessment of B_{12} Status

There are several B_{12} assessment techniques such as serum or plasma B_{12}, TCII, homocysteine (tHcy), serum or urinary methylmalonic acid (MMA), and mean corpuscular volume (MCV). Each assessment technique has varying levels of accuracy in terms of diagnosing B_{12} depletion or deficiency. Serum or plasma B_{12} and TCII are direct measure of B_{12}, and tHcy and MMA are metabolites affected by B_{12} status. MCV is an assessment of the erythrocytes volume. TCII and MMA are the most accurate, whereas serum or plasma B_{12} and MCV are believed to be unreliable.[3,7] TCII can be used to assess B_{12} depletion, whereas MMA indicates B_{12} deficiency.[15] Microbiological assay used to measure serum B_{12} level, although commonly used, is especially unreliable because it measures both active B_{12} forms along with inactive B_{12} analogues.

According to Herbert,[15] low serum B_{12} is a relatively late indicator of B_{12} deficiency whereas TCII indicates an early B_{12} depletion. Serum or plasma B_{12} level includes a total circulating B_{12} from all B_{12} carriers, including TCII and haptocorrin. At any given time, at least 80% of B_{12} is bound to haptocorrin. Haptocorrin values reflect body stores of B_{12}, including B_{12} stored in the liver. According to Herbert,[15] all cells have receptors for TCII whereas only the liver has receptors for haptocorrin. Thus, depletion of B_{12} in some cells may develop before hepatic cells are depleted and before serum B_{12} level shows a subnormal value. Serum assessment may not show low values until hepatic stores are depleted, which consequently will affect haptocorrin B_{12} values. Haptocorrin has a half-life of approximately 240 hours whereas TCII only 6 minutes.[15] For the reasons mentioned above, TCII is considered more useful in detecting B_{12} depletion early.[16] Thus, using TCII instead of serum B_{12} assessment will help in detecting B_{12} depletion before stores of B_{12} from the liver are depleted.

It is generally accepted that a level of serum B_{12} that is less than 200 pg/mL (150 pmol/L) is considered to be B_{12} deficiency. However, many people show symptoms of B_{12} deficiency with higher levels of serum B_{12}. Herbert[15] suggested using <300 pg/mL (222 pmol/L) as the cutoff value for deficiency. A value of <35 pmol/L is most often used when assessing B_{12} status with TCII.

Methylmalonic acid and tHcy are elevated when B_{12} status is compromised. Both of these metabolites are considered early signs of biochemical B_{12} deficiency.[16] Elevated tHcy (>12 μmol/L) may also indicate folate or pyridoxine deficiency. Thus, additional tests have to be done to determine B_{12} status. MMA is considered one of the most reliable assessments of B_{12}. The Institute of Medicine set the normal urine MMA value as 0.58 to 3.56 μmol/mmol creatinine.[7] Different cutoff values for serum MMA have been used, including 210, 260, 271, 370, and higher.[16] MMA is a especially sensitive marker of B_{12} deficiency; however, MMA, and to a lesser degree tHcy are affected by the renal function and thus in patients with kidneys insufficiency, using them to assess B_{12} status may be inaccurate.

Normal MCV is between 80 and 96 fL. Higher values indicate a megaloblastic anemia. MCV is affected not only by B_{12} level but also iron and folate status. Iron deficiency causes microcytic erythrocytes and thus decreases MCV, whereas folate deficiency causes megaloblastic erythrocytes. This is why assessment of MCV is also not reliable to assess B_{12} status because it also reflects the status of folate and/or iron. In addition, MCV values may be normal with high folate intake even in the presence of B_{12} deficiency.

Several researchers suggested using more than one assessment methods such as TCII and MMA or tHcy and MMA to evaluate B_{12} status. Although MMA is considered the most sensitive marker of B_{12} status, it is also the most expensive and this makes is less attractive for clinical use than TCII or tHcy.[16]

Deficiency

Although once thought that B_{12} deficiency is rare and unlikely to develop, except in strict vegetarians, studies conducted in the past few decades showed that this view has been based on the incorrect assumption, that it takes many years for the deficiency to develop.[17-21] These studies showed that B_{12} deficiencies are common in both developing and wealthier countries. Deficiency is particularly high among the elderly and vegetarians, but it is also prevalent among pregnant women and children, especially in less developed countries.[17] Studies showed that B_{12} deficiency is common among people of European and African descents, Indian subcontinent, Central America, and South America. In Asia, B_{12} deficiency is less prevalent except in vegetarians.[18]

B_{12} deficiency may occur as a result of malabsorption due to a gastrointestinal condition, inadequate intake, lack of synthesis of TCII, a genetic defect of methylmalonyl coenzyme A mutase, or nitrous oxide poisoning. Malabsorption may have 2 primary causes: inadequate or complete inability of synthesis of IF and/or inadequate synthesis of or synthesis of weak hydrochloric acid, essential in activating pepsinogen to pepsin that digests dietary R-proteins. Although malabsorption due to the above-listed conditions is most prevalent among the elderly, it may occur at any age among people who have undergone gastrectomy such as in ulcer disease, gastric bypass for obesity, patients with celiac disease, Crohn's disease, and those receiving chemotherapy. Deficiency due to inadequate intake has been reported in strict vegetarians, lacto-ovo-vegetarians, people following a macrobiotic diet, those who follow some form of raw food diets, children with

regular disturbance in feeding behavior especially coupled with increased metabolic demand, and alcoholics. Also, mild B_{12} deficiency is common in many countries with relatively low intake of meat and animal products such as Guatemala, Mexico, or Venezuela.[19-21]

The rate of B_{12} deficiency reported in studies depends largely on several factors, including the assessment method and cutoff points used. Deficiency prevalence may have been underestimated in studies that used serum B_{12} assessment for the reason described earlier.

According to the National Health and Nutrition Examination Survey data reported by Pfeiffer et al,[22] 7% of the US population 3 years and older has elevated MMA (>370 nmol/L). The rate of B_{12} deficiency among elderly (>60 years) Latinos from California participating in the SALSA study was 27.6% and an additional 40.3% had marginal B_{12} status.[23] Twenty-three percent of the elderly from Georgia were diagnosed with B_{12} deficiency in a study led by Johnson[24] (MMA > 271 nmol/L).

Using MMA as the indicator of B_{12} status, B_{12} deficiency has been reported in between 55% to slightly more than 85% in children adhering to macrobiotic diets (deficiency defined as >430 nmol/L), 41% of adolescents formerly on macrobiotic diet (deficiency defined as >290 nmol/L), 31% of adult lacto-ovo- or lacto-vegetarians who were taking supplements (deficiency defined as >260 nmol/L), 47% of Chinese vegetarian women 55 years and older (deficiency defined as >0.4 µmol/L), and 68% among Buddhist and Taoist monks living in Hong Kong (deficiency defined as >0.4 µmol/L).[19,20,25,26] Deficiency among lacto-ovo-vegetarians and vegans from Germany and the Netherlands was between 68% and 90%.[25-29] B_{12} deficiency is relatively high among people in the developing world such as countries in South America with relatively low meat and animal products intake. Some studies showed a deficiency rate of 50% or higher.[19,20]

In rare cases, deficiency may also occur because of lack of or inadequate synthesis of TCII. TCII is the protein carrier of B_{12} to all cells in the body, including transport of B_{12} from intestines via the portal vein. Also, in even rarer cases, a genetic defect of methylmalonyl coenzyme A mutase, essential in the breakdown of some amino acids, is a cause of deficiency. In addition, all people who undergo a surgery under anesthesia should be screened for B_{12} deficiency because nitrous oxide causes irreversible oxidation of cobalt, deactivating B_{12}.[30]

Table 1.

Health Conditions/Symptoms Associated With Vitamin B_{12} Deficiency.

Types	Results	Clinical Manifestations
Hematological	Pernicious anemia Megaloblastic anemia Leukopenia Thrombocytopenia	Large, immature red blood cells, lower than the normal amount of white blood cells, abnormally low number of platelets in the bloodstream
Neurological	Demyelination Neuropathy	Nervousness Striking behavioral changes Paranoia Tingling Fatigue Paralysis Memory loss Numbness
Psychiatric		Irritability Personality change Mild memory impairment Depression Psychosis
Health conditions		Multiple sclerosis Parkinson disease Alzheimer's disease Dementia Cancer Occlusive vascular diseases Congenital heart defects Osteoporosis Failure to thrive Premenstrual syndrome Neural tube defects

B_{12} deficiency symptoms can be grouped into several categories, including hematological, neurological, and psychiatric. Specific symptoms of B_{12} deficiency in each of the above categories are listed in Table 1. Although description of symptoms of B_{12} deficiency has been widely published, deficiency of B_{12} is very often misdiagnosed. This is because these symptoms mimic symptoms of other health conditions such as Alzheimer's disease, spinal cord compression, amyotrophic lateral sclerosis, diabetic peripheral neuropathy, alcoholic peripheral neuropathy, and congestive heart failure.[31,32] The classic B_{12} deficiency symptoms include synthesis of large, immature, oblong-shaped erythrocytes (megaloblasts) and myelin deterioration in both central and peripheral nervous systems.[32] Megaloblastic anemia leads

to symptoms such as weakness, fatigue, lightheadedness, tachycardia, angina, and pale skin.[7] However, high intakes of folate and iron-deficiency anemia can mask the megaloblastic symptoms, making assessment of erythrocytes volume an unreliable assessment tool for B_{12} status. The deterioration of myelin may progress to axonal degeneration and even axonal death, which leads to neuropathy.

Toxicity

Hydroxocobalamin taken in high dose has been associated with acne-like dermatological effect.[33] Cyanocobalamin does not exhibit similar association. It has been hypothesized that the dermatological effect seen after ingestion of hydroxocobalamin was due to degradation products of hydroxocobalamin rather than hydroxocobalamin itself.[33] A tumor-promoting effect of high doses of B_{12} has been suggested based on studies on rats.[34] However, neither carcinogenic nor mutagenic effects of B_{12} were confirmed in human studies even when mega doses of B_{12} were used for a very long time.[35-37] No symptoms of toxicity were reported among patients with pernicious anemia, patients on dialysis, and geriatric patients. Even in studies where a dose of 1000 to 2000 times greater than the RDA for several months to a few years were used, no symptoms of toxicity were reported.[35-37] Thus, it is commonly accepted belief that B_{12} does not appear to have any toxic side effects even when mega doses of B_{12} are used as supplements, in parenteral administrations, or in injections.[7,38]

Discussion

B_{12} deficiency is a prevalent nutrient deficiency resulting from a variety of factors, including inadequate intake and absorption. In the United States, to prevent B_{12} deficiency, people 50 years and older are recommended to take supplements of this vitamin, as stated in the Dietary Guidelines for Americans document.[39] It is, however, reasonable to assume that the majority of people in this age-group are not aware that they should be taking B_{12} supplements. Furthermore, although some vegetarians may be aware of the risk, many do not supplement B_{12} for a variety of reasons, including common misconceptions such as that a deficiency develops only in strict vegetarians after many years of following such a diet.[40] In fact, the popularity of this misconception is not exclusive to some vegetarians. It has been disseminated in some scientific publications and even in a statement published on the National Library of Medicine's Web site.[41] B_{12} deficiency is widespread and is associated with many adverse health outcomes from mild, such as forgetfulness and lack of energy, to severe, such as loss of smell, neural tube defects, multiple sclerosis, Alzheimer's disease, and inability to work.

Although taking a B_{12} supplement has been shown to be effective in both prevention and treatment of B_{12} deficiency, there is little effort on the part of public health officials to promote its use. Also, as already indicated, some groups of vegetarians resist taking B_{12} supplements. Furthermore, the experience with promoting the use of folic acid–containing supplements to prevent neural tube defects has shown that promotion campaigns are largely ineffective.[42] Many years since such recommendation was first issued by the Center for Disease Control and Prevention, women are still unsure of what is the best time to use them, the dose, and the form of folic acid.[42] This experience indicates it may take a very long time to increase awareness of the prevalence of B_{12} deficiency and any efforts to combat this epidemic may be ineffective.

Some scientists have proposed fortification of flour with B_{12}.[22] Fortification of flour with folic acid contributed to an increase in folic acid content of red blood cells, decreased total homocysteine and consequently, a reduction in rates of neural tube defects.[22] It is reasonable to assume that this preventive measure would have the same effect in terms of reduction in B_{12} deficiency and its associated symptoms. Oakley and Tulchinsky[43] suggested that B_{12} deficiency symptoms would disappear if mandatory fortification of flour with B_{12} was implemented in Europe. However, the benefits of fortifying products such as flour with B_{12} can only be accomplished if adequate dose of B_{12} were used. Different doses of B_{12} fortification have been proposed, including 1, 10, and 20 µg per 100 g of flour.[44-46] Although these proposed doses range from about 50% of the US RDA to almost 9 times higher than that RDA, only about 1% of the crystalline form of B_{12} can absorbed. Carmel[47] suggested that fortification of flour with just 1 to 10 µg will be ineffective in preventing B_{12} deficiency among the most vulnerable groups such as the elderly. In fact, Carmel suggested that if a total intake of B_{12} is less than 100 µg in the elderly, it would neither prevent the deficiency nor replenish the body's stores of B_{12}. Thus, for the best benefit, flour would have to be fortified with a dose of 100 µg or higher per 100 g. Cyanocobalamin is the most stable of all B_{12} forms and thus it is the best candidate to use in fortification. AJLM

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