Vitamin B12

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Related nutrients/biomarkers: cobalt; folate deficiency also produces megaloblastic anemia and elevated serum total homocysteine but the two deficiencies can be distinguished using biomarker assays. There is controversial evidence that high folic acid intake from supplements can exacerbate B12 deficiency. Vitamin B6 and riboflavin are cofactors in the same metabolic pathways as B12.

Importance of vitamin B12 for health

Vitamin B12, or cobalamin, is essential for human health and survival. It consists of a corrin ring with a cobalt atom in the center. The cobalt atom is bound to cyano or hydroxyl groups forming cyanocobalamin or hydroxocobalamin, the forms commonly used as supplements. In cells the usual forms are methylcobalamin and 5'-deoxyadenosyl cobalamin.

A major review of B12's metabolism, function and requirements provides useful information relevant to all sections of this document (1). The European Food Safety Authority (EFSA) also has a recent review focused on B12 requirements (2).

Vitamin B12 serves as a cofactor for two enzymes:

I. methylmalonyl CoA mutase, which converts methylmalonyl CoA to succinyl CoA, an intermediate in the oxidation of fatty acids and the breakdown of some amino acids and

II. methionine synthase, which converts homocysteine to methionine in a reaction with folate as a cofactor.

Metabolic functions of vitamin B12 include:

- Synthesis of red blood cells
- Nerve function
- Brain function
- Prevention of neural tube defects
- Prevention of growth and cognitive impairment in infants and young children

Vitamin B12 is an essential nutrient which must be consumed in animal source foods, fortified foods or supplements.

Dietary B12 is bound to proteins from which it is released by gastric acid, binds to haptocorrin from saliva, then travels to the ileum along with intrinsic factor secreted by the stomach. In the ileum B12 is released from haptocorrin and binds to intrinsic factor, and is absorbed as a B12-intrinsic factor complex by a receptor-mediated active process. B12 is transported in blood bound to transcobalamin and serum holotranscobalamin is an alternative biomarker for recent B12 intake. At intakes above about 2.5 µg/day, the recommended intake (RDA), the B12-intrinsic factor complex becomes saturated so that the percent absorbed, by passive transport, falls to only ≈1% from doses above ≈25 µg/day. Nevertheless, high doses (500-1000 µg) are often used to maintain B12 status in people with no intrinsic factor due to pernicious anemia, or the elderly, as absorption of 1% of these high doses is sufficient. Alternatively, high doses of hydroxocobalamin or cyanocobalamin (1000 µg) can be given by intramuscular injection weekly, then monthly, if necessary.

Risks of deficiency:

Globally the prevalence of vitamin B12 depletion and deficiency is high, primarily due to low intake of animal source foods which are the only natural source of the vitamin (1).

Low consumption of animal products occurs in many low-income groups, and when such foods are avoided for cultural, religious or ethical reasons. Elderly persons may be more likely to be vitamin B12 deficient due to their having lower gastric acid and intrinsic factor, sometimes due to chronic, long-term infection with *Helicobacter pylori*. The autoimmune disease pernicious anemia, which can occur at any age but has a higher prevalence in the elderly (2 to 4% after age 60 years), will also cause

deficiency because in this disease there is a lack of the intrinsic factor required for B12 absorption. Other conditions which impair B12 absorption include Crohn disease, celiac disease, ileal bypass or resection, and gastric bypass or removal of part of the stomach. Unfortunately tests for B12 malabsorption are difficult and the only methods available today are based on increase in serum holotranscobalamin after doses of B12 (the Cobasorb test) or administration of very low dose 14C-B12 (3, 4). B12 from supplements or fortified foods will be better absorbed than B12 from food if gastric acid is low, so this is sometimes a recommendation for elderly people.

Infants born to mothers deficient in B12 are born with low liver stores of the vitamin and then exposed to low levels in their mother's milk; there are many case studies of infants presenting with severe growth and mental retardation around 3-4 months of age (5). High dose supplements to the infant resolve some but not always all of these problems.

Clinical symptoms of deficiency include changes in biomarkers in blood and urine (see table below), followed by weakness and fatigue, neurological impairments such as peripheral neuropathy, reduced myelination and conductivity of neurons, megaloblastic anemia, brain atrophy and dementia (1). Deficiency has to be relatively severe to produce megaloblastic anemia, so anemia is more common in strict vegetarians, malabsorptive conditions, and the elderly than in persons with a chronically low dietary intake. In fact, the functional consequences of subclinical cobalamin deficiency (called SCCD), which is generally diagnosed by biochemical tests, are not well defined. Importantly, one does not have to be a strict vegetarian to become depleted in B12; as the amount of any animal source food in the diet falls, then poorer B12 status will result (1).

Risks of excess:

There are no known risks of consuming large amounts of vitamin B12 so consequently there are no upper limits to intake recommendations. When intakes are high absorption of a dose is <1% due to saturation of the intestinal receptors.

Drug interactions: Medications that affect vitamin B12 absorption or metabolism include:

- Inhibitors of gastric acid secretion such as proton pump inhibitors (PPIs) and histamine receptor 2 antagonists, because gastric acid is needed to release the vitamin from food.

- Treatment of diabetes with metformin lowers serum B12. Initially this was thought to be a sign of B12 deficiency, but later research indicates that metformin causes the vitamin to accumulate in the liver.

- Chronic exposure to the anesthetic nitrous oxide, which oxidizes methylcobalamin.

Biomarkers for measuring vitamin B12 intake and status

Biomarker	Analysis type	Sample	Benefits	Intricacies
Vitamin B12	Direct analysis	Serum, plasma	Relatively sensitive and specific Reflects long term B12 status	Deficiency occurs after several years of low intake, but within months if malabsorption
Methylmalonic acid	Indirect analysis	Serum, urine	Reflects the adequacy of B12 for function of methylmalonyl mutase	Expensive instrument set-up needed (LC-MS). Increased if poor kidney function so often requires serum creatinine analysis as well.
Holo-transcobalamin	Indirect analysis	Serum	Best reflects recent intake and absorption	Gives similar prevalence of deficiency to serum B12 in surveys
Homocysteine	Indirect analysis	Plasma	Relatively sensitive but also increased by folate, B2 and B6 deficiency	
2 to 4 of the above biomarkers combined	Direct/indirect analysis	Serum, plasma	The combined B12 status marker (cB12) is more specific than single biomarkers (6)	Multiple analyses and instruments needed.

Direct analysis of vitamin B12

Serum or plasma: Serum or plasma vitamin B12 has been measured using commercially available competitive protein binding immunoassays (CPBA). Alternatively, a microbiological assay using *L. delbruekii* subsp. *lactis* ATCC™ 4797 or 7830 can be used for B12 analysis.

Human milk: In the last 10 years, two methods have been described to accurately analyze human milk vitamin B12 by modifying existing CPBAs to remove interfering amounts of haptocorrin from the milk sample.

Functional B12 assessment

Plasma or urine methylmalonic acid using mass spectrometry reflects the adequacy of B12 for methylmalonyl CoA mutase function.

Neurological assessment including nerve conduction velocity, vibratory sensation, abnormal gait, peripheral neuropathy and MRI which detects loss of white matter in the spinal cord and brain.

Methods

Consult the reference list for examples of published methods for each assay. Links to each method can be found on https://www.open-global.kcl.ac.uk/vitamin-B12/

- Chemiluminescence immunoassay for B12 in serum or plasma (7, 8).
- Microbiological assay for B12 in serum or plasma (9).
- Monoclonal antibody assay for serum holotranscobalamin (10).
- LC-MS/MS or GC-MS/MS for serum (preferred), RBC, or urine methylmalonic acid (11-13).
- HPLC with fluorescence detection for plasma homocysteine (14).
- Competitive enzyme immunoassay for B12 in human milk (15, 16).

Accreditation schemes

ARUP Laboratories: A National Reference Laboratory. This is a non-profit laboratory at the University of Utah which is a global leader in clinical and pathological test development. It provides accreditation for vitamin B12 (and folate); methylmalonic acid in serum, plasma or urine; and total homocysteine in serum or plasma. https://ltd.aruplab.com/testing/licensure-accreditations

LabCorp, based in North Carolina, USA, also provides accreditation services for:

- vitamin B12, https://www.labcorp.com/test-menu/36696/vitamin-bsub12-sub;
- methylmalonic acid in serum or plasma https://www.labcorp.com/test-menu/31226/methylmalonic-acid-serum-or-plasma.

Alternatively, please see the OpeN-Global page on laboratory accreditation: https://open-global.kcl.ac.uk/accreditation/

Technical assistance

For questions on methods of vitamin B12 assessment or for technical assistance, please contact the OpeN-Global team at https://open-global.kcl.ac.uk/contact/ or write to:

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Useful links

The Biomarkers of Nutrition for Development (BOND) review on vitamin B12 provides a major review of B12's metabolism, function and requirements, and provides useful information relevant to each page of this OpeN-Global fact-sheet:
https://academic.oup.com/jn/article/148/suppl_4/1995S/5219149

The European Food Safety Authority (EFSA) also has a recent review focused on B12 requirements:
https://efsa.onlinelibrary.wiley.com/doi/abs/10.2903/j.efsa.2015.4150

US National Academies Press IOM Dietary reference intakes for thiamine, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline, 2000:
http://nationalacademies.org/hmd/reports/2000/dietary-reference-intakes-for-thiamin-riboflavin-niacin-vitamin-b6-folate-vitamin-b12-pantothenic-acid-biotin-and-choline.aspx

US National Institutes of Health, Health Professionals Fact Sheet on vitamin B12:
https://ods.od.nih.gov/factsheets/VitaminB12-HealthProfessional/

World Health Organisation statement: Conclusions of a WHO Technical Consultation on folate and vitamin B12 deficiencies:
https://www.who.int/nutrition/publications/micronutrients/FNBvol29N2supjun08.pdf

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