Vitamin B12 (Cyanocobalamin)

Synonyms:
Cobalamin, Coenzyme B12, Adenosylcobalamin, AdoCbl, cobamamide, Antipernicious-anemia factor, Castle's extrinsic factor, animal protein factor.

Chemistry:
The structure of vitamin B12 is based on a corrin ring, which has two of the pyrrole rings directly bonded. The central metal ion is Co (cobalt). Four of the six coordinations are provided by the corrin ring nitrogens, and a fifth by a dimethylbenzimidazole group. The sixth coordination partner varies, being a cyano group (-CN) (cyanocobalamin), a hydroxyl group (-OH) (hydroxycobalamin), a methyl group (-CH3) (methylcobalamin) or a 5'-deoxyadenosyl group (5-deoxyadenosylcobalamin).

Food:

<table>
<thead>
<tr>
<th>Food</th>
<th>µg/100g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef liver</td>
<td>65</td>
</tr>
<tr>
<td>Crab</td>
<td>27</td>
</tr>
<tr>
<td>Blue mussel</td>
<td>8</td>
</tr>
<tr>
<td>Steak</td>
<td>5</td>
</tr>
<tr>
<td>Coalfish</td>
<td>3.5</td>
</tr>
<tr>
<td>Cheese (Camembert)</td>
<td>3</td>
</tr>
<tr>
<td>Eggs</td>
<td>1 – 3</td>
</tr>
</tbody>
</table>

(Souci, Fachmann, Kraut)

Main functions:
• Coenzyme-function in intermediary metabolism, especially in cells of the nervous tissue, bone marrow and gastrointestinal tract
• Essential growth factor
• Formation of blood cells and myelin sheaths
• Regeneration of folate
• Involved in the production of melatonin (controls the release of many hormones in the body and is involved with the sleep/wake cycle)

For scientific sources, please contact info.nutritionscience@dsm.com.
Vitamin B12 (Cyanocobalamin)

Vitamin B12, which is only found in foods of animal origin, is the largest and most complex of all the vitamins. In the body, the vitamin supports the development of red blood cells and DNA, maintains healthy nerve cells and releases energy from food. Mild deficiencies of B12 are not uncommon in elderly people, usually due to poor diet or because individuals have less stomach acid which is needed to absorb the vitamin.
Functions

Vitamin B12 is necessary for the formation of blood cells, nerve sheaths and various proteins. It is therefore essential for the prevention of pernicious anemia and neurological disturbances. It is also involved in fat and carbohydrate metabolism and is essential for growth. In humans, vitamin B12 functions primarily as a cofactor in intermediary metabolism. Two enzymes are dependent on vitamin B12:

1. Methionine synthase which converts homo-cysteine to methionine
2. Methylmalonyl CoA mutase which converts methylmalonyl CoA to succinyl CoA

In its methylcobalamin form vitamin B12 is the direct cofactor for methionine synthase, the enzyme that recycles homocysteine back to methionine. In addition, methionine synthase and vitamin B12 are involved in the production of the active forms of folate and low vitamin B12 may disrupt folate metabolism.

Absorption and body stores

Vitamin B12 from food sources is bound to proteins and only released by an adequate concentration of hydrochloric acid in the stomach. Free vitamin B12 is then immediately bound to glycoproteins originating from the stomach and salivary glands. This glycoprotein complex protects vitamin B12 from chemical denaturation. Gastrointestinal absorption of vitamin B12 occurs in the small intestine by an active process requiring the presence of an intrinsic factor, another glycoprotein, which the gastric parietal cells secrete after being stimulated by food. The absorption of physiological doses of vitamin B12 is limited to approximately 2µg/dose. The vitamin B12 intrinsic factor complex is then absorbed through phagocytosis by specific ileal receptors. Once absorbed, the vitamin is transferred to a plasma-transport protein which delivers the vitamin to target cells. A lack of intrinsic factor prevents vitamin B12 absorption. If this is untreated, potentially irreversible neurological damage and life- threatening anemia develops (see Deficiency).

Regardless of dose, approximately 1% of vitamin B12 is absorbed by passive diffusion, so this process becomes quantitatively important at pharmacological levels of exposure. Once absorbed, vitamin B12 is stored principally (60%) in the liver. The average B12 content is approximately 1.0 mg in healthy adults, with 20 – 30 µg found in the kidneys, heart, spleen and brain. Estimates of total vitamin B12 body content for adults range from 0.6 to 3.9 mg with mean values of 2 – 3 mg. The normal range of vitamin B12 plasma concentrations is 150 – 750 pg/ml, with peak levels achieved 8 – 12 hours after ingestion.

Excretion of vitamin B12 is proportional to stores and occurs mainly by urinary and fecal routes. Vitamin B12 is very efficiently conserved by the body, with 65 – 75% re-absorption in the ileum of the 0.5 – 5 µg excreted into the alimentary tract per day (mainly into the bile). This helps to explain the slow development (over several years) of deficiency states in subjects with negligible vitamin B12 intake, such as vegans. Patients with a lack of intrinsic factor (i.e. pernicious anemia) will rapidly develop vitamin B12 deficiency as they can only absorb small amounts of the vitamin.

Dietary sources

Vitamin B12 is produced exclusively by microbial synthesis in the digestive tract of animals. Therefore, animal protein products are the main source of vitamin B12 in the human diet. Other sources include fish, eggs and dairy products. In foods, hydroxy-, methyl- and 5'-deoxy- adenosyl-cobalamins are the main cobalamins present. Bacteria in the intestine synthesize vitamin B12, but not in areas where absorption occurs. Some foods are also fortified with vitamin B12.
Measurement

Measurement of vitamin B12 in plasma is routinely used to determine deficiency but may not be a reliable indication in all cases. In pregnancy, for example, tissue levels are normal, but serum levels are low. Vitamin B12 can be measured by chemical, microbiological or immunoassay isotope dilution methods. Microbiological assays, which are widely used for blood and tissue samples, are sensitive but non-specific.

Serum cobalamin concentration is often determined by automated immunoassays using intrinsic factor as a binding agent. These assays have mainly replaced microbiological methods.

Data in literature about vitamin B12 serum concentration varies. However, values under 110 – 150 pmol/L are considered to reflect deficiency, whereas values over 150 – 200 pmol/L represent an adequate status. Major vitamin B12-dependent metabolic processes include the formation of methionine from homocysteine, and the formation of succinyl coenzyme A from methylmalonyl coenzyme A. Thus, apart from directly determining vitamin B12 concentration in the blood, elevated concentrations of both methylmalonic acid (MMA) and homocysteine may indicate a vitamin B12 deficiency. Vitamin B12 concentrations can also be measured using the novel biomarker holoTC.

Stability

Vitamin B12 is stable to heat, but slowly loses its activity when exposed to light, oxygen and acid or alkali-containing environments. Loss of activity during cooking is due to the water solubility of vitamin B12 rather than its destruction.

Physiological interactions

- Absorption of cobalamins is impaired by alcohol and vitamin B6 deficiency. Furthermore, a number of drugs reduce the absorption of vitamin B12, and supplementation with the affected nutrient may be necessary:
  - Antibiotics (e.g. chloramphenicol)
  - Anti-diabetics (e.g. metformin and phenformin)
  - Anti-epileptic drugs
  - Anti-gout medication (Colchicine)
  - Stomach medication (H2 receptor antagonists, Proton pump inhibitors)
  - Nitrous oxide (anesthetic)
  - Oral contraceptives
  - Tuberculostatics (Para-aminosalicylic acid)
  - Several anticonvulsants – phenobarbitone, primidone, phenytoin and ethylphenacemide – can alter the metabolism of cobalamins in the cerebrospinal fluid and lead to neuropsychic disturbances. Several substituted amide, lactone and lactam analogues of cyanocobalamin compete with binding sites on intrinsic factor and lead to depressed absorption of the vitamin. Nitrous oxide (anesthetic) also interferes with cobalamin metabolism.

Recommended daily intakes (RDI) *

<table>
<thead>
<tr>
<th>Group</th>
<th>Life stage</th>
<th>Dose/day**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants</td>
<td>&lt;6 months</td>
<td>0.4 µg (AI)</td>
</tr>
<tr>
<td>Infants</td>
<td>7 – 12 months</td>
<td>0.5 µg (AI)</td>
</tr>
<tr>
<td>Children</td>
<td>1 – 3 years</td>
<td>0.9 µg</td>
</tr>
<tr>
<td>Children</td>
<td>4 – 8 years</td>
<td>1.2 µg</td>
</tr>
<tr>
<td>Children</td>
<td>9 – 13 years</td>
<td>1.8 µg</td>
</tr>
<tr>
<td>Adults</td>
<td>&gt;14 years</td>
<td>2.4 µg</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>14 – 50 years</td>
<td>2.6 µg</td>
</tr>
<tr>
<td>Breastfeeding</td>
<td>14 – 50 years</td>
<td>2.8 µg</td>
</tr>
</tbody>
</table>

* Institute of Medicine (2001)
** Adequate intake (AI)

If not otherwise specified, this table presents Recommended Dietary Allowances (RDIs). Allowable levels of nutrients vary depending on national regulations and the final application.
Deficiency

Vitamin B12 deficiency affects 10 – 15% of individuals over the age of 60.

Deficiency of vitamin B12 leads to defective DNA synthesis in cells, which affects the growth and repair of all cells. The tissues most affected are those with the greatest rate of cell turnover, e.g. those of the hematopoietic system. This can lead to megaloblastic anemia (characterized by large and immature red blood cells) and neuropathy, with numerous symptoms including: glossitis, weakness, loss of appetite, loss of taste and smell, impotence, irritability, memory impairment, mild depression, hallucination, breathlessness (dyspnea) on exertion, tingling and numbness (paraesthesia). Vitamin B12 deficiency can also lead to hyperhomocysteinemia, a possible risk factor for occlusive vascular disease. Low vitamin B12 has been associated with a variety of chronic diseases of aging such as dementia and cognitive impairment, cardiovascular disease (CVD) and osteoporosis.

The symptoms of vitamin B12 deficiency are similar to those of folic acid deficiency, the major difference being only that vitamin B12 deficiency is associated with spinal cord degeneration. If folic acid is used to treat vitamin B12 deficiency, anemia may be alleviated but the risk of damage to the nervous system remains. Nervous dysfunction associated with vitamin B12 can be irreversible and potentially life threatening if left untreated. It is therefore essential to diagnose the deficiency accurately before starting therapy.

Deficiency is usually caused as a result of vitamin B12 malabsorption. Without intrinsic factor, absorption is not possible and a severe and persistent deficiency develops that cannot be prevented by the usual dietary intakes of vitamin B12.

Groups at risk

- Vegetarians
- The elderly
- Alcoholics
- People with:
  - pernicious anemia (autoimmune disease, chiefly affects people post middle age)
  - food-bound vitamin B12 malabsorption (in patients receiving long-term treatment with certain drugs, elderly patients with gastric atrophy, patients with atrophic gastritis)
  - after gastrectomy
  - after ingestion of corrosive agents with destruction of gastric mucosa
  - lesions of the small bowel; bacterial overgrowth; patients with small intestinal defects; inborn errors of cobalamin metabolism etc.
  - pancreatic insufficiency
  - AIDS

Pernicious anemia:

Pernicious anemia is the classical symptom of B12 deficiency, but it is actually the end stage of an autoimmune inflammation of the stomach, resulting in destruction of stomach cells by the body’s own antibodies. Anemia is a condition in which red blood cells do not provide adequate oxygen to body tissues. Pernicious anemia is a type of megaloblastic anemia.

Gastric atrophy:

Gastric atrophy is a chronic inflammation of the stomach resulting in decreased stomach acid production. As this is necessary for the release of vitamin B12 from the proteins in food, vitamin B12 absorption is reduced.

Reducing disease risk: therapeutic use

Pernicious anemia

Pernicious anemia patients are traditionally treated with intramuscular injections of vitamin B12; large oral doses of the vitamin are also effective but require lifetime therapy. When used alone, oral doses of at least 150 µg/day are necessary, although single weekly oral doses of 1,000 µg have proved satisfactory in some cases.

Hyperhomocysteinemia

Homocysteine appears to be a nerve and vessel toxin, promoting mortality and CVD as well as stroke, Alzheimer’s disease, birth defects, recurrent pregnancy loss, and eye disorders. Keeping homocysteine at levels associated with lower rates of disease requires adequate vitamin B12, folic acid and vitamin B6 intake.
Cancer
Vitamin B12 deficiency may lead to an elevated rate of DNA damage and altered methylation of DNA. These are obvious risk factors for cancer. In a recent study, chromosome breakage was minimized in young adults by supplementation with 700 µg of folic acid and 7 µg of vitamin B12 daily in cereals for two months.

Recommended Daily Intake (RDI)
The US Institute of Medicine (IOM) recommends that anyone over 50 years should consume most of their vitamin B12 from fortified foods or supplements. During pregnancy, it is recommended that women consume 2.6 µg/day and up to 2.8 µg/day during breastfeeding to cover the additional requirements of the fetus/infant. The Committee on Nutrition of the American Academy of Pediatrics recommends a daily vitamin B12 intake of 0.15 µg/100 kcal energy intake for infants and preadolescent children. Other authorities have suggested intakes of 0.4 – 0.5 m µg (0 – 1 year of age), 0.9 – 1.8 µg (1 – 10 years of age) and 2.4 µg (>10 years). The 'average' Western diet probably supplies 3 – 15 µg/day, but this can range from 1 – 100 µg/day.

Safety
Large intakes of vitamin B12 from food or supplements have caused no toxicity in healthy people. No adverse effects have been reported from single oral doses as high as 100 mg and chronic administration of 1 mg (500 times the RDI) weekly for up to 5 years. Moreover, there have been no reports of carcinogenic or mutagenic properties, and studies to date indicate no teratogenic potential. The main food safety authorities have not set a UL for vitamin B12 because of its low toxicity.

Supplements and food fortification
The principal form of vitamin B12 used in supplements is cyanocobalamin. It is available in the form of injections and as a nasal gel for the treatment of pernicious anemia. Cyanocobalamin is also available in tablet and oral liquid form for vitamin B-complex, multivitamin and vitamin B12 supplements. Vitamin B12 is widely used to enrich cereals and certain beverages. Fortification with vitamin B12 is especially important for products aimed at people with a low dietary vitamin B12 intake, such as vegans.

Production
Vitamin B12 is produced commercially from bacterial fermentation, usually as cyanocobalamin.

History

1824 The first case of pernicious anemia and its possible relation to disorders of the digestive system is described by Combe.

1855 Combe and Addison identify clinical symptoms of pernicious anemia.

1925 Whipple and Robscheft-Robins discover the benefits of consuming liver in regenerating blood in anemic dogs.

1926 Minot and Murphy report that a diet rich in large quantities of raw liver restores the normal level of red blood cells in patients with pernicious anemia. Liver concentrates are developed and studies on the presumed active principle(s) ('antipernicious anemia factor') are initiated.

1929 Castle postulates that two factors are involved in the control of pernicious anemia: an ‘extrinsic factor’ in food and an ‘intrinsic factor’ in normal gastric secretion. Simultaneous administration of these factors causes red blood cell formation which alleviates pernicious anemia.

1934 Rickes, Smith and Parker, working separately, isolate a crystalline red pigment which they name vitamin B12.

1948 Pierce and team isolate two crystalline forms of vitamin B12 that are equally effective in combating pernicious anemia. One form is found to contain cyanide (cyanocobalamin), while the other is not (hydroxocobalamin).

1949 Hodgkin and colleagues establish the molecular structure of cyanocobalamin and its coenzyme forms using X-ray crystallography.

1955 Eschenmoser and colleagues in Switzerland and Woodward and team in the US synthesize vitamin B12 from cultures of certain bacteria/fungi.

1955 Total chemical synthesis of vitamin B12 by Woodward.

1973 West shows that injections of vitamin B12 dramatically benefit patients with pernicious anemia.

1995 Whipple, Minot and Murphy are awarded the Nobel Prize for Medicine for their work in the treatment of pernicious anemia.