

Risk Assessment

Vitamin B₁₂

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General Information

Chemistry

Vitamin B₁₂ (cobalamin, Cbl) is a water-soluble vitamin and a member of a family of related molecules known as corrinoids which contain a corrin nucleus made up of a tetrapyrrolic ring structure. The centre of the tetrapyrrolic ring nucleus contains a cobalt ion that can be attached to methyl, deoxyadenosyl-, hydroxo- or cyano- groups.

Natural occurrence

Vitamin B₁₂ originates from bacteria, fungi and algae, and is present in virtually all animal tissues. Plants contain no vitamin B₁₂ beyond that derived from microbial contamination.

Occurrence in food, food supplements and medicines

Major dietary sources of vitamin B₁₂, mainly in the forms of methyl, deoxyadenosyl- and hydroxocobalamin, include meat (e.g. > 0.1 mg/kg in lamb), particularly liver (> 0.1 mg/kg) and fish (e.g. 0.03-0.1 mg/kg in salmon, 0.01-0.03 mg/kg in tuna). Hydroxocobalamin and, in particular, cyanocobalamin are synthetic forms used in vitamin supplements, pharmaceuticals and in the fortification of food. Methyl cobalamin has been used therapeutically outside the UK, for example, in Japan.

Recommended amounts

The RNI for vitamin B₁₂ in adults in the UK is 0.0015 mg/day (COMA, 1991). There is no increment required during pregnancy but there is a recommended increment of 0.0005 mg/day for breast feeding mothers.

Analysis of tissue levels and vitamin B₁₂ status

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. Various other plasma markers have been identified (including methylmalonic acid, homocysteine, holotranscobalamin, anti-intrinsic factor antibodies) and methods devised (Schilling test, cobalamin absorbance test, serum gastrin deoxyuridine suppression test) to distinguish different causes of deficiency.

Brief overview of non-nutritional beneficial effects

Results of studies in humans have suggested that large doses of vitamin B₁₂ (particularly methyl cobalamin) may influence biological rhythms and thus may be beneficial in the treatment of sleep disorders. Vitamin B₁₂ has also been reported to increase light sensitivity by affecting melatonin secretion. Vitamin B₁₂, in combination with folic acid, has been suggested to be beneficial in certain disorders, such as idiopathic osteoarthritis and vitiligo.

Function

Vitamin B₁₂ serves as a cofactor to at least two enzymes, methionine synthase and methylmalonyl CoA mutase. Methionine synthase is pivotal in one-carbon metabolism, being crucial in the synthesis of the universal methyl donor S-adenosyl methionine and in the cellular import and metabolism of folate. Methylmalonyl CoA mutase converts L-methylmalonyl CoA to succinyl CoA and is important in even-chained fatty acid synthesis.

Deficiency

Dietary deficiency is rare in younger people living in the community but occurs more frequently in older people, particularly those living in institutional environments. Individuals adhering to vegan diets may also be at risk. Deficiency is mostly attributable to inherited or acquired defects resulting in malabsorption or the impairment of transport of the vitamin within the body. Deficiency impacts on the haematopoietic and nervous systems. Associated diseases include megaloblastic anaemia and neuropathies typically sub-acute combined degeneration of the spinal cord. Vitamin B₁₂ deficiency can lead to moderate hyperhomocysteinaemia, a possible risk factor for occlusive vascular disease.

Oral supplements are indicated prophylactically where there is a likelihood of deficiency in those whose gastrointestinal function is normal e.g. in individuals who are strict vegetarians. Inherited and acquired disorders relating to vitamin B₁₂ malabsorption are usually treated by repeated injection. However, oral administration of very high doses of vitamin B₁₂ has been shown to be effective in the treatment of pernicious anaemia.

Interactions

Steroid drugs, such as prednisone, have been reported to increase the absorption of vitamin B₁₂ in patients with pernicious anaemia. Excessive alcohol consumption and some drugs may decrease absorption of vitamin B₁₂. Oral co-administration with ascorbic acid may result in destruction of vitamin B₁₂. Concurrent administration of chloramphenicol may lead to antagonism of the haematopoietic response to vitamin B₁₂.

Absorption and bioavailability

Vitamin B₁₂ requires intrinsic factor (IF), secreted mainly from the gastric parietal cells, to ensure adequate absorption at normal dietary intake levels. Thus the absorption of physiological doses of vitamin B₁₂ is limited to approximately 0.0015 – 0.002 mg/dose or meal, due to saturation of the uptake system. Regardless of dose, approximately 1.2% of vitamin B₁₂ is absorbed by passive diffusion and consequently this process becomes quantitatively important at pharmacological levels of exposure. Protein binding in certain foods may reduce the bioavailability of the vitamin, particularly in individuals with impaired gastric acid and/or digestive enzyme secretion. The different forms of crystalline cobalamin appear to be absorbed or retained to different extents, depending on the dose. Differences are most apparent at low doses.

Ingested vitamin B₁₂ is released from the food matrix by the action of digestive enzymes and gastric acid and becomes bound to salivary haptocorrin-binding proteins. As the pH rises further along the gut, and under the influence of pancreatic enzymes, vitamin B₁₂ is released from the salivary haptocorrin and becomes complexed with intrinsic factor (IF). The cobalamin-IF complex binds to a specific cell wall

receptor of the ileal enterocyte and is internalised by endocytosis. Once inside the cell, the IF is degraded and the liberated vitamin is converted to the methyl or the deoxyadenosyl form, is bound to transcobalamin II (TC II) binding protein and then exported into the portal blood. In the general circulation, most cobalamin is bound to transcobalamin I (TC I) but the majority of cobalamin available for uptake into the tissues is that bound to TC II.

Distribution and metabolism

Vitamin B₁₂ is distributed into the liver, bone marrow and virtually all other tissues, including the placenta and breast milk of nursing mothers. The liver is the predominant storage site for vitamin B₁₂.

Uptake into cells occurs through receptor mediated endocytosis involving specific TC II cell wall receptors. Once inside the tissues/cells, the complex is degraded by the lysosomes, and the released cobalamin is metabolised either to methyl-cobalamin in the cytosol, where it binds to methionine synthase, or to deoxyadenosyl-cobalamin in the mitochondria, where it binds to methylmalonyl CoA mutase.

Excretion

Excretion occurs mainly via the faeces and urine, but also through the shedding of skin cells. Excretion is very slow, with significant enterohepatic cycling.

Toxicity

Human data

There are a few case reports of adverse effects associated with ingestion of vitamin B₁₂, either as a supplement, or following the consumption of yeast extract products, which also contain cyanocobalamin. Five cases of allergic reactions were reported, three of which were recurrences of symptoms in individuals who had been previously exposed to cobalamin by the parenteral route. One further case reported the occurrence of a skin eruption that resembled acne rosacea. Vitamin B₁₂ exposures were generally not specified.

No adverse effects were reported in an experiment designed to determine the uptake of single oral doses of cyanocobalamin (up to 100 mg). However, only three participants were administered the very high doses.

Oral studies have been conducted to investigate the effects of vitamin B₁₂ on pernicious anaemia. However, although no adverse effects are apparent, these studies are not relevant to the general population since absorption of vitamin B₁₂ is reduced in this condition. The effect of high oral-dose cyanocobalamin on plasma homocysteine levels in healthy females of child-bearing age and the benefits of cyanocobalamin in patients with seasonal affective disorder have been investigated. No adverse effects related to treatment were reported in any study including those in which individuals received up to 4.5 mg/day cyanocobalamin for 14 days, 2.0 mg/day cyanocobalamin for up to one year or 1.0 mg/day cyanocobalamin for several years. Less information is available following the oral administration of the hydroxocobalamin form of vitamin B₁₂. However, no adverse effects were reported

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in individuals administered 0.3 mg/day for up to 12 months. No adverse effects were reported in a controlled study in which 125 individuals received 6.0 mg/day methylcobalamin for up to 12 weeks.

Adverse reactions (not specified) were reported in one of 16 and in one of 23 oligozoospermia patients given 6 or 12 mg/day methyl cobalamin, respectively, for 16 weeks, presumably via the oral route. However, this study was not controlled.

Animal data

The data-base on the oral toxicity of vitamin B₁₂ in laboratory animals is limited. Doses of 1.5 to 3.0 mg/kg bw by intraperitoneal and subcutaneous administration were found to be acutely toxic in mice (CNS effects; convulsions, cardiac and respiratory failure and ultimately death). However, much higher doses (≥ 5 g/kg bw) cyanocobalamin appeared to be tolerated by mice following oral administration. There is no evidence relating to vitamin B₁₂ and teratogenicity or adverse effects on fertility or post-natal development.

Carcinogenicity and genotoxicity

There is no evidence suggesting that vitamin B₁₂ is carcinogenic or genotoxic *in vitro* or *in vivo*. However, although data are not consistent, there is some limited evidence to suggest that high doses of vitamin B₁₂ may have tumour promoting activity.

Mechanisms of toxicity

No data have been identified.

Dose-response characterisation

No relevant data have been identified.

Vulnerable groups

No vulnerable groups have been identified.

Genetic variations

No genetic variations have been identified.

Studies of particular importance in the risk assessment

(For full review see <http://www.food.gov.uk/science/ouradvisors/vitandmin/evmpapers> or the enclosed CD).

Waife et al., 1963

As part of a non-controlled supplementation trial in patients with pernicious anaemia (n=27) no adverse reactions were reported in individuals receiving 0.3 mg hydroxocobalamin/day for up to one year.

Berlin et al., 1968

No adverse effects attributable to vitamin B₁₂ (cyanocobalamin) were recorded in a long-term clinical trial of 64 patients with pernicious anaemia, and other types of vitamin B₁₂ deficiency given a daily oral dose of 0.5 mg rising to 1.0 mg of cyanocobalamin (without intrinsic factor) for 10 to 70 months (42 patients received treatment for over 4 years). However, as noted previously, systemic absorption in these patients is limited.

Juhlin and Olsson, 1997

One hundred patients with vitiligo were treated with oral folic acid (5 mg) and vitamin B₁₂ (1.0 mg cyanocobalamin) twice daily, for up to 12 months. There were no reports of adverse effects. However, 27/100 and 48/100 participants had stopped taking the supplements after 1 – 2 months and 3 – 6 months, respectively but reasons for withdrawal were not stated.

Takahashi et al., 1999

As part of a double-blind study to assess the therapeutic effect of methyl cobalamin on sleep-wake disorder, patients were administered either 6.0 (n=21) or 0.03 mg/day (n=27) methylcobalamin for 8 weeks. The lower dose group was considered as a control group because ethical permission was not granted for the inclusion of a placebo control group. There was no report of any adverse effects. The route of administration was not clear from the information given. Data were not available for all patients at the end of 8 weeks (two of the 6.0 mg/day dose group and three of the 0.03 mg/day group).

Exposure assessment

Total exposure/intake¹⁸:

Food	Mean: 0.0062 mg/day 97.5 th percentile: 0.020 mg/day (1986/7 NDNS)
Supplements	up to 3.0 mg/day (Annex 4)

Estimated maximum intake: 0.020 + 3.0 = 3.0 mg/day

No potential high intake groups have been identified.

¹⁸ The survey data do not distinguish the different forms of vitamin B₁₂. Dietary vitamin B₁₂ is mainly in the methyl, deoxyadenosyl and hydroxocobalamin forms. Hydroxo- and particularly cyanocobalamin are the forms usually present in dietary supplements.

Risk assessment

Vitamin B₁₂ is a water-soluble vitamin. At physiological doses, as occurs in food, the amount absorbed is largely limited (approximately 0.002 mg/meal) by the capacity of the intrinsic factor-wall receptor uptake system. At pharmacological levels of dosing, diffusion becomes more important as the route of absorption. Vitamin B₁₂ present in excess of the binding capacity of the liver, plasma and other tissues is excreted by glomerular filtration.

It is generally accepted that ingested vitamin B₁₂ (cobalamin) has a very low toxicity in humans. Most available documented data are either in the form of case reports of possible vitamin B₁₂-associated adverse effects or from clinical trials or supplementation studies designed primarily to investigate potential beneficial effects. The latter generally involve the use of the cyanocobalamin or methylcobalamin forms of vitamin B₁₂ and do not always specifically report an absence of adverse effects.

The animal toxicity database for vitamin B₁₂ is very limited. Doses of 1.5 to 3.0 mg/kg bw by intraperitoneal and subcutaneous administration were found to be acutely toxic in mice (CNS effects; convulsions, cardiac and respiratory failure and ultimately death). However, much higher doses (≥ 5 g/kg bw) cyanocobalamin appeared to be tolerated by mice following oral administration.

ESTABLISHMENT OF GUIDANCE LEVEL

There are insufficient data from studies in humans and animals to set a Safe Upper Level for vitamin B₁₂.

Clinical studies have reported no adverse effects following administration of up to 6.0 mg/day of methylcobalamin for several weeks and up to 1.0 mg/day cyanocobalamin for several years. Clinical trials and supplementation studies involving up to 100 individuals to investigate the beneficial effects of oral cyanocobalamin have not reported any treatment-related adverse reactions following doses of 0.3 to 4.5 mg for periods ranging from 14 days to several years. Cyanocobalamin is the type of vitamin B₁₂ most frequently included in supplements in the UK. The study by Juhlin and Olsson (1997), supported by the absence of an identified hazard and widespread clinical experience with oral and parenteral treatment, suggests that supplemental intakes of 2.0 mg cyanocobalamin/day should not produce any adverse effects and this intake can be used for guidance purposes. This is equivalent to 0.034 mg/kg bw/day in a 60 kg adult. No uncertainty factor is needed because human data from large numbers of individuals are available. However, it should be noted that this figure has been established in particular subgroups of the population, i.e. vitiligo sufferers and those treated for pernicious anaemia, and may not be completely applicable to the general population.

References

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