12. Vitamin B_{12}

Physiology and metabolism

Metabolic functions

The term vitamin B_{12} covers the two forms active in the body – 5'-deoxyadenosyl-cobalamin and methylcobalamin – and a number of other cobalt-containing corrinoids which by being converted to the active forms have the same nutritional effect.

Two enzymes in man have a requirement for vitamin B_{12} [1]. Methionine synthase uses methylcobalamin and is involved in the channelling of carbon units from amino acids such as serine to remethylate homocysteine to methionine. This remethylation is necessary because all tissues use methionine as a source of methyl groups and as a consequence generate significant amounts of homocysteine which needs to be remethylated. Methylmalonyl CoA mutase, which uses 5'-deoxyadenosyl-cobalamin, is necessary in the metabolism of propionyl CoA, which arises either from the catabolism of certain amino acids or the oxidation of odd-chain fatty acids.

Absorption

Dietary vitamin B_{12} is bound by a glycoprotein called "intrinsic factor" secreted by the parietal cells of the stomach. The resultant intrinsic factor – vitamin B_{12} complexes pass to the ileum where they are absorbed. About three quarters of an oral dose of 0.5 µg is absorbed while the amount absorbed from 1.0 µg is half [1]. Intrinsic factor-mediated absorption seems to have an upper limit of about 1.5 µg per meal irrespective of how much vitamin B_{12} is presented to it.

Excretion

It has been calculated that the biliary secretion of vitamin B_{12} is around 0.5 µg per day [1]. Perhaps less than 20 % or 0.1 µg of this is lost through non-reabsorption [2].

Vitamin B_{12} is very well stored. Its half-life in humans has been measured in a number of studies as being of more than one year to almost four years [3].

Deficiency

Vitamin B_{12} levels in most diets appear to be adequate, the exception being strict vegetarian or vegan diets. As will be discussed later, individuals or communities on such diets have well documented lower than normal blood levels of vitamin B_{12} and some studies show biochemical evidence of deficiency. This is easily understood since plant food does not contain any vitamin B_{12} unless contaminated with microorganisms [1]. Individuals among the elderly and the poor may also have an increased risk of deficiency where their diet is low in animal produce.

By far the biggest cause of vitamin B_{12} deficiency is impairment of absorption [4]. This arises most often from destruction of the parietal cells of the stomach by autoantibodies, resulting in diminution or absence of secretion of intrinsic factor. Autoantibodies are also frequently produced against intrinsic factor itself, some of which render it incapable of binding vitamin B_{12}, with more rarely other antibodies permitting binding of vitamin B_{12} but preventing uptake of the complex by the ileum [1]. These three autoimmune conditions are called pernicious anaemia (PA).

Deficiency, when it occurs, has two general consequences: arrest of cell replication and neurological damage [1]. The former manifests itself clinically in the more rapidly dividing cells such as those of the marrow, the immune system, the skin and the gastrointestinal tract. Anaemia is the most obvious clinical sign. Appropriate treatment with the vitamin completely reverses all the effects on cell replication. The neurological lesion presents first as paraesthesia with a tingling sensation in the fingers and/or toes with or without numbness. Untreated, this will progress to a peripheral neuropathy and ataxia and an overt demyelination of the spinal cord called sub-acute combined degeneration. Psychiatric manifestations such as confusion, depression, agitation and delusions may also be present. Treatment with vitamin B_{12} appears to completely redress the latter manifestations. The peripheral neuropathy will also improve but the demyelination of spinal cord is essentially irreversible.

It is now widely accepted that, as described originally in the 5-methyl-tetrahydrofolate trap hypothesis [5], the arrest of cell division seen in vitamin B_{12} deficiency results from an impairment in the proper utilisation of the folate cofactors involved in the synthesis of DNA and RNA in replicating cells. One of these cofactors, 5-methyltetrahydrofolate, requires to be demethylated by vitamin B_{12}-dependent methionine synthase for its further metabolism. In the absence of vitamin B_{12} the other cellular folates needed to synthesise DNA and RNA become metabolically trapped in this form resulting in a 'pseudo' folate deficiency

in such cells, which causes an anaemia identical to that seen in simple folate deficiency.

The neurological lesion is thought to be due to the inability to utilize 5-methyltetrahydrofolate to remethylate homocysteine to methionine [6,7]. The latter supplies methyl groups for the synthesis of proteins and lipids in myelin and other nerve structures.

Higher and undesirable intakes

Daily ingestion of high amounts of vitamin B_{12}, of even one or two orders of magnitude above normal dietary levels, does not cause any obvious side effects even over a prolonged period. However, nutritionally inactive vitamin B_{12} analogues can be found in some vitamin preparations and increasing their intake may not be without risk. A daily intake greater than 200 µg should be discouraged.

Requirements

Criteria of vitamin B_{12} nutritional adequacy

A number of methods have been used to assess vitamin B_{12} status or to detect its deficiency.

The serum concentration of the vitamin can be determined; the results vary somewhat according to the procedure used, but most laboratories would regard concentrations of less than 100 ng/L as indicating deficiency, 100-150 ng/L as possibly deficient, with values above 150 ng/L being taken as normal [1,8]. These ranges cannot however be regarded as wholly reliable as there is some overlapping.

In B_{12} deficiency, the methylmalonic acid concentrations in urine and plasma might be expected to increase. The recent availability of methods sensitive enough to estimate methylmalonate in plasma has revealed a very good correlation between raised methylmalonate concentration and the presence of clinical disease, but there can be some overlap with the reference range [9].

Traditionally the presence of a macrocytic anaemia has been considered to be the most usual way of picking up subjects with vitamin B_{12} deficiency. However very

deficient subjects can sometimes have normal concentrations of haemoglobin and no increase in mean corpuscular volume [10].

While they are difficult to perform, neurological assessments, preferably using newer methods such as evoked response studies, are really the only way of determining the presence or absence of neurological damage due to vitamin B_{12} deficiency.

Individually these tests may not be wholly reliable but the use of two or more gives useful information in the B_{12} status of individuals and groups that can be used to make recommendations on B_{12} intake.

Adults

A number of approaches have been used to calculate requirements.

Studies have been made on the response of patients with pernicious anaemia, who have no contribution from dietary vitamin B_{12}, to daily parenteral doses. They indicate that 0.3 µg/d is not quite adequate [11] but 0.5 µg/d is [12,13]. These patients however will secrete some B_{12} in the bile which will not be reabsorbed and so will be lost from the enterohepatic circulation. Dietary values for normal subjects would therefore need to be adjusted downwards for that reason, but also upwards to allow for incomplete absorption from the diet. If it could be said that these would balance out, one might expect a daily intake of 0.3 µg vitamin B12 to be too little and 0.5 µg to be adequate on the evidence of these studies using mainly haematological criteria.

The amount of B_{12} in the diet of strict vegetarians has been investigated. Studies on sizeable populations indicated that while a mean intake of 0.26 µg/d might provide inadequate stores, no deficiency was detected [14]. Similar studies indicated that smaller groups got by on intakes ranging from 0.3 to 0.5 µg/d [15,16]. This work depended mainly on abnormal haematological findings to detect abnormal states. While some studies assessed neurological function, this is not easy to do and perhaps subtle changes that were present might not have emerged. While earlier studies that related intake to well being did not look for biochemical evidence of deficiency, from subsequent studies on similar strict vegetarian groups it seems very likely that biochemical deficiency, as detected by elevated urine methylmalonic acid levels, exists on diets containing these low levels of vitamin B_{12} [17,18]. It appears that biochemical deficiency would exist with a risk of eventual neurological dysfunction on intakes less than 0.5 µg/d.

Two studies have been based on vitamin turnover. They made a number of assumptions about various factors and came up with mean daily requirements of between 0.25 and 1.0 µg/d [19] and of 1.3 µg/d [20].

At levels of 0.5 µg/d that are likely to be consumed by subjects on strict vegetarian diets, there is no evidence of haematological or neurological dysfunction, but there is biochemical abnormality. Interpretation of many of the studies of the type cited above depends very heavily either on the absence of apparent clinical problems in populations or upon the apparent adequacy of the treatment. These studies regarded the absence of haematological abnormalities as being an assurance of general health, and it is difficult to rule out neurological damage because it is so much more difficult to assess [21]. Examination of the early literature makes it clear that many people with apparently normal haematology were developing irreversible neurological damage. More recent studies suggest that this circumstance is much more common than is usually appreciated.

It therefore seems prudent to put forward a mean requirement towards the high end of the scale: 1.0 µg/d.

Pregnancy

There seems to be no risk of signs of clinical deficiency developing in a pregnant woman, even on a diet low in vitamin B_{12}. The major cause for concern is the effect of such low intakes on the developing embryo and on progeny that are subsequently breast-fed. It is clear that children of strict vegetarian mothers who were subsequently breast-fed, and then went on to vegetarian diets themselves, are at serious risk not just of biochemical evidence of deficiency as indicated by elevated methylmalonic acid but also of decreased growth rates [18]. Such low status has been shown to lead to overt signs of neurological damage, which in time would become irreversible [22]. There thus seems no doubt that women on strict vegetarian diets should be encouraged to increase their intake of vitamin B_{12} during pregnancy and that this advice should, as a precaution, be extended to all women. Unlike folate, there is no evidence for a high rate of turnover of vitamin B_{12} in pregnancy. However to allow for transfer of some of the vitamin to the fetus, an additional 0.2 µg/d is recommended.

Lactation

During lactation, loss of the vitamin in milk depends to a large extent upon the mother's vitamin B_{12} status [23]. It is difficult to assess how much if any of this loss needs to be reinstated in women who have normal stores. However if the values in milk fall below about 0.37 µg per day, evidence of biochemical deficiency begins

to be seen in breast-fed infants [17] (who probably had very inadequate stores of vitamin B_{12} to begin with). It would thus seem prudent to try to replace at least 0.37 µg per day. If one estimates that about three quarters of a dose is absorbed at this level, this requires an increment of 0.5 µg/d during lactation.

Children

Infants born to women with a diet very low in B_{12}, and thus with very poor stores of the vitamin, needed 0.37 µg/d to cure biochemical deficiency as evidenced by methylmalonic acid excretion [17]. A PRI for infants 6-11 months is set at 0.5 µg/d.

In the absence of specific studies, values for children have been calculated from those for adults on the basis of energy expenditure.

The elderly

There appears to be no increased requirement for vitamin B_{12} with age [24]. While a decrease in vitamin B_{12} status as measured by serum levels has been reported in some studies as age advanced, it is almost certainly due to an increased prevalence of malabsorption due either to the autoimmune disease pernicious anaemia or to atropic gastritis [25].

Summary

(amounts in µg/d, based on a relative molecular mass of 1355)

Adults	**Average Dietary Requirement**	1.0
	Population Reference Intake (Mean requirement + 2SD, assuming a coefficient of variation in this case of 20%)	1.4
	Lowest Threshold Intake (Mean requirement - 2SD)	0.6
Pregnancy	Plus	0.2
Lactation	Plus	0.5

Population Reference Intakes for other age groups

(amounts in µg/d, based on a relative molecular mass of 1355)

6 - 11 m	0.5
1 - 3 y	0.7
4 - 6 y	0.9
7 - 10 y	1.0
11 - 14 y	1.3
15 - 17 y	1.4

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