

Vitamin B₁₂ deficiency

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Abstract | Vitamin B₁₂ (B12; also known as cobalamin) is a B vitamin that has an important role in cellular metabolism, especially in DNA synthesis, methylation and mitochondrial metabolism. Clinical B12 deficiency with classic haematological and neurological manifestations is relatively uncommon. However, subclinical deficiency affects between 2.5% and 26% of the general population depending on the definition used, although the clinical relevance is unclear. B12 deficiency can affect individuals at all ages, but most particularly elderly individuals. Infants, children, adolescents and women of reproductive age are also at high risk of deficiency in populations where dietary intake of B12-containing animal-derived foods is restricted. Deficiency is caused by either inadequate intake, inadequate bioavailability or malabsorption. Disruption of B12 transport in the blood, or impaired cellular uptake or metabolism causes an intracellular deficiency. Diagnostic biomarkers for B12 status include decreased levels of circulating total B12 and transcobalamin-bound B12, and abnormally increased levels of homocysteine and methylmalonic acid. However, the exact cut-offs to classify clinical and subclinical deficiency remain debated. Management depends on B12 supplementation, either via high-dose oral routes or via parenteral administration. This Primer describes the current knowledge surrounding B12 deficiency, and highlights improvements in diagnostic methods as well as shifting concepts about the prevalence, causes and manifestations of B12 deficiency.

Vitamin B₁₂ (B12; also known as cobalamin) is one of eight B vitamins and its role in cellular metabolism is closely intertwined with that of folate, another B vitamin (FIG. 1). Since the discovery and characterization of B12 more than 60 years ago and the recognition of its central role in preventing the serious disease known as pernicious anaemia, much has become known about B12 deficiency^{1,2}. Pernicious anaemia originally acquired its appropriate eponym because of the ultimately fatal haematological and devastating neurological manifestations of the disease and was later shown to be caused by autoimmune destruction of gastric parietal cells and their product, intrinsic factor (also known as gastric intrinsic factor), which is required for B12 absorption. Previously considered to be a nutritional deficiency disease that was largely caused by malabsorption of the vitamin and restricted to older people, particularly those of North European descent, B12 deficiency is now considered to be a problem of global dimensions, frequently caused by dietary inadequacy, particularly among children and in women of reproductive age³.

The effects of B12 deficiency are mainly seen in the blood and nervous system. The classic manifestations of B12 deficiency were first identified in pernicious anaemia, the cause of which was then unknown^{4,5}. Since then,

the spectrum has shifted considerably, starting with the renewed recognition that neurological manifestations (such as sensory and motor disturbances (particularly in the lower extremities), ataxia, cognitive decline leading to dementia and psychiatric disorders) often predominate and can frequently occur in the absence of haematological complications⁶. In addition, the identification of subtler degrees of B12 deficiency⁷, made possible by the introduction of assays for the metabolites methylmalonic acid (MMA) and homocysteine in clinical practice^{6,8} (FIG. 1), has broadened the landscape of what might be attributable to B12 deficiency, but has opened a Pandora's box of controversy regarding what could be considered actual, clinical B12 deficiency as opposed to states of metabolic inadequacy of the vitamin^{7,9}. Progression from normalcy to clinical deficiency passes through a stage of inadequacy during which biochemical evidence of B12 insufficiency in the form of increased blood and tissue levels of MMA and homocysteine and declining levels of the portion of B12 bound to transcobalamin (known as holotranscobalamin) precede the appearance of any morbid manifestations of deficiency. This condition has also been referred to as 'subclinical' B12 deficiency and is associated with low or marginal B12 levels¹⁰. Individuals with biomarkers indicating a subclinical deficiency are

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particularly challenging for the clinician because it is not known whether these individuals will progress to overt deficiency or remain in a chronic but stable low B12 status of no clinical relevance^{11–13}.

In this Primer, we give a comprehensive description of the epidemiology of B12 deficiency and dietary recommendations to prevent its occurrence, as well as the pathophysiology, diagnosis, preventive and public health issues, and management surrounding the condition.

Epidemiology**Risk factors**

In higher-income countries, the major risk factor for developing B12 deficiency (BOX 1) is the well-characterized autoimmune disease pernicious anaemia, which is caused by a lack of production of intrinsic factor by gastric parietal cells that is needed for the intestinal absorption of B12 and eventually leads to the development of anaemia and/or severe neurological symptoms. Pernicious anaemia can affect people of all ages, but its incidence rises with age^{1,5,14}. Conservative estimates indicate that pernicious anaemia affects 2–3% of individuals ≥ 65 years of age^{1,14}. In addition, important risk factors are gastrointestinal surgery, such as gastric bypass or removal of the terminal ileum, which compromise the absorption of B12. However, in low-income countries, B12 deficiency is largely due to a low intake of B12-rich foods of animal origin, but possibly also to gastrointestinal infections and infestations, and host–microbiota interactions¹⁵.

Contributing risk factors include *Helicobacter pylori* infection, intestinal bacterial overgrowth, poor food intake, alcoholism, smoking and long-term use of drugs, such as proton pump inhibitors, histamine H2 receptor antagonists and metformin. Various diseases, including malaria, HIV infection and tuberculosis¹⁶ might

contribute to deficiency through a combination of factors. Conversely, although not the cause of the primary disease, concomitant B12 deficiency may affect the progression of the particular condition, for example, in HIV infection¹⁷. Surprisingly, in one study, slum dwellers had better B12 status than urban dwellers, possibly related to poorer hygiene in the slums, which putatively exposes individuals to ingestion of B12-containing microorganisms¹⁵. Both individual-level and population-level factors (such as socioeconomic status, religion, cultural practices and public health policies) contribute to the general health status and B12 status of a population.

Prevalence

General population. Clinical B12 deficiency, with classic haematological or neurological manifestations, is relatively uncommon. Low or marginal B12 status, in the absence of overt haematological or neurological impairments, is much more common, particularly in populations with a low intake of B12-rich, animal-sourced foods¹⁸. B12 status in the United States has been extensively assessed in the National Health and Nutrition Examination Survey (NHANES). Using NHANES data from 1999 to 2004 (REF. 19), the prevalence of B12 status defined as low was estimated to be 2.9%, 10.6% or 25.7% based on serum B12 cut-off values of <148, <200 and <256 pmol per litre, respectively. Using these cut-off values, the prevalence of low B12 status increased with age from young adults (19–39 years of age) to older adults (≥ 60 years of age), and was generally higher in women than in men (prevalence of 3.3% versus 2.4% with a serum B12 level of <148 pmol per litre, respectively). Using instead increased levels of MMA as a functional indicator of B12 status¹⁹ (TABLE 1), the prevalence of low B12 status was 2.3% or 5.8% based on cut-off values of >0.376 and >0.271 μmol per litre, respectively. The prevalence of increased levels of MMA increased with age and was not different between men and women. Notably, only 50–75% of participants in NHANES with low levels of serum B12 had increased levels of MMA¹⁹. Individuals with low levels of total serum B12 and increased levels of MMA might better reflect the true prevalence of actual B12 deficiency (defined biochemically) than those with only one of the markers outside the cut-off limits. Data from NHANES 2003–2006 indicate that the consumption of B12-fortified foods and supplements improved B12 status²⁰.

National studies from various countries have shown that prevalence estimates for low B12 status (<148 pmol per litre) and marginal deficiency (148–221 pmol per litre) far exceed those in the United States, particularly in South America, Africa and Asia (FIG. 2). Notably, a high prevalence of low B12 status is not confined to older adults, with some countries exceeding >40% prevalence in different subpopulations (children, young adults, women of childbearing age, pregnant women and older adults).

B12 status, as assessed by blood levels of B12 and its biomarkers, varies throughout an individual's lifetime, as does the prevalence of B12 deficiency. When interpreting these markers it is, therefore, important to take age and physiological circumstances of the individual into consideration.

Elderly individuals. As noted, B12 deficiency is more prevalent among older people, generally increasing beyond 60 years of age^{21–23}. Underlying causes of deficiency are diverse and extend well beyond the 2–3% prevalence of pernicious anaemia²⁴ (BOX 1). Among older people, 10–15% have subclinical B12 deficiency^{12,19}, which can often, but not always, be normalized with B12 therapy^{19,25,26}. The prevalence is even higher in the ‘oldest-old’, with reports of 23–35% of individuals over 80 years of age having B12 deficiency²⁷. Variable susceptibility across different population and racial groups has also been reported^{14,23}.

Pregnancy and lactation. Pregnancy and lactation alter maternal B12 status in a manner that facilitates the transfer of B12 to the fetus and infant. Profound physiological and anatomical changes occur in virtually every organ system during pregnancy with considerable consequences on biochemical markers, thus complicating the evaluation of micronutrient status and limiting the use of established reference ranges determined in non-pregnant women²⁸. Most markers of B12 status (circulating levels of total B12, holotranscobalamin, MMA and homocysteine) are lower during pregnancy than pre-pregnancy

levels. Total B12 levels in the mother gradually decrease during pregnancy^{28–30}, whereas circulating levels of holotranscobalamin decrease or remain relatively stable (FIG. 3). Homocysteine and MMA levels increase during the latter part of pregnancy compared with the first trimester, which may be indicative of a degree of metabolic intracellular B12 depletion in pregnant women, despite the fact that both homocysteine and MMA are lower than the established cut-off levels defining deficiency in non-pregnant women^{28,30–32}. Indeed, this increase depends on maternal B12 status²⁸ and is lower in women taking B12 supplements³¹. This indicates a need for specific reference ranges based on B12-replete (supplemented to normal levels) pregnant and lactating women. Owing to these changes, the true prevalence of B12 deficiency in pregnancy is difficult to quantify, but is reported to occur in <10% (in Canada and Brazil)^{33,34} to >70% (in parts of India and Turkey) of pregnant women^{35–37}.

In one study, postpartum, maternal circulating B12 levels were reported to be significantly higher than in non-pregnant women³¹. This might represent a physiological adaptation to enhance mobilization of maternal B12 stores for transfer to the infant through increasing levels in breast milk (BOX 2).

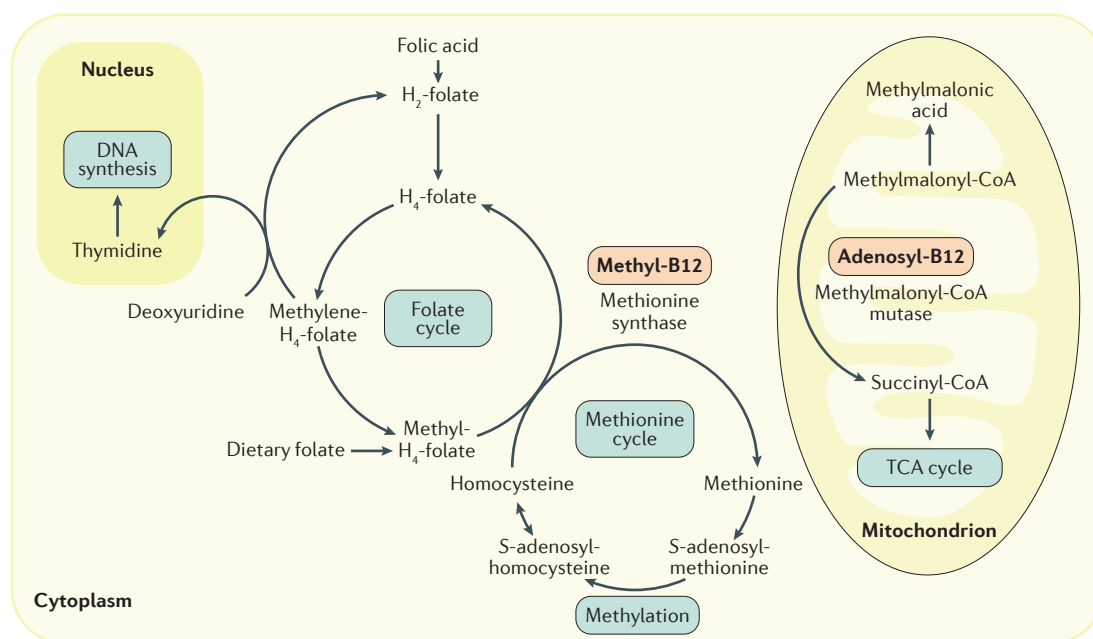


Figure 1 | Vitamin B₁₂ and folate metabolism and function. Vitamin B₁₂ (B12) and folate are required for the methionine synthase reaction in which a methyl group is transferred from methyltetrahydrofolate (methyl-H₄-folate; also known as levomefolic acid) to homocysteine by methionine synthase, with methyl-B12 as a coenzyme to form methionine. The resulting H₄-folate can then be returned to the folate pool and made available for the generation of methylene-H₄-folate, the form required for *de novo* synthesis of thymidine, which is essential for DNA replication and repair. Hence, either folate or B12 deficiency results in the same biochemical perturbation in thymidine synthesis and DNA replication. In the case of B12 deficiency, folate is ‘trapped’ in the unusable methyl-form^{5,194}. B12 is also involved in the conversion of methylmalonyl-CoA (methylmalonic acid bound to coenzyme A) to succinyl-CoA by the enzyme methylmalonyl-CoA mutase with adenosyl-B12 as a cofactor; succinyl-CoA is a major intermediary of the tricarboxylic acid (TCA) cycle. In B12 deficiency, substrates of both B12-dependent reactions accumulate, which leads to increased levels of methylmalonic acid and homocysteine in the plasma. A combination of low levels of B12 and increased levels of folate was associated with higher concentrations of methylmalonic acid and total plasma homocysteine^{156,157}. Major complications of B12 deficiency are megaloblastic anaemia, as a result of inhibition of DNA synthesis, and neurological manifestations.

Infancy and childhood. Infancy, childhood and adolescence are times of rapid growth, during which demand for B12 is high and B12 status undergoes marked changes^{38,39}. Low maternal B12 status, extended breastfeeding and a low intake of animal food after weaning are major risk factors for B12 deficiency. During the first months of life, B12 levels decrease, whereas homocysteine and MMA levels increase, with the lowest B12 concentrations and the highest homocysteine and MMA levels seen between 4 and 6 months in exclusively breastfed infants^{39–41}. From 6 months, serum B12 levels increase and peak between 3 and 7 years of age and then decrease throughout adolescence. Plasma homocysteine levels decrease and remain low (<6 µmol per litre) up to 7 years of age, and then start to increase; the median plasma MMA levels decrease after 6 months and remain low throughout childhood (<0.26 µmol per litre)³⁹.

During the first 2 years of life, the homocysteine level is a reliable marker of B12 status, whereas in older children and adults in high-income countries, homocysteine levels mainly reflect folate status in non-folic-acid-fortified populations³⁹. In breastfed infants (BOX 2), MMA levels are inversely related to B12 levels, but the MMA concentrations are relatively high throughout the B12 distribution than in older children³⁹. The higher MMA levels may be due to intestinal absorption of propionate and other MMA precursors that are produced by intestinal bacteria or by degradation of odd chain fatty acids that are present in breast milk^{39,42}.

A biochemical profile with low levels of B12 and high levels of homocysteine and MMA, which is indicative of impaired B12 functional status, is seen in more than two-thirds of mainly breastfed Norwegian infants of 6 weeks to 4 months of age⁴³, a finding that is also observed in

other infant populations^{41,44}. Intervention studies with a single intramuscular dose of 400 µg of hydroxy-B12 given at 6 weeks have demonstrated that it is possible to improve this profile, with a 39% reduction in homocysteine levels and a 66% reduction in MMA levels, indicating that the profile commonly seen in breastfed infants does indeed reflect a modifiable state of B12 inadequacy and not simply organ immaturity^{43,45}.

In studies from Pune, India, there was a precipitous fall in circulating B12 concentrations from 6 to 12 and 18 years of age, and deficiency (<150 pmol per litre) was found in 16%, 24% and 58% at those ages, respectively (C.Y., unpublished observations). B12 concentrations were associated with a genetic risk score for B12-related morbidity based on eight independently associated single-nucleotide polymorphisms in genes encoding proteins involved in B12 metabolism, maternal vitamin concentrations in pregnancy and weight gain during childhood (C.Y., unpublished observations). In addition, a strong intra-familial correlation for B12 status has been reported, indicating that the 'family environment' (comprising socioeconomic factors, food habits, hygiene, and religious and cultural practices) is important. A cross-sectional study showed similar intra-familial associations of B12 status in Amazonian children (4.5% deficient)⁴⁶.

Mechanisms/pathophysiology

B12 deficiency relates to a series of pathophysiological mechanisms that can occur in infancy, childhood, adolescence and adult life, all of which can affect the B12 supply, the demand or both. Cellular deficiency in B12 is caused by inadequate intake, malabsorption, chemical inactivation, or inherited disruption of either B12 transport in the blood or intracellular metabolism^{1,4,5}.

Inadequate intake and bioavailability

Microorganisms (that is, bacteria and archaea) are the ultimate source of B12 in nature, and, in conventional diets, B12 is exclusively available from animal food sources, such as meat, liver, fish, eggs and dairy products. The daily B12 requirement of 2–3 µg is easily met in those who consume large amounts of animal products or who take supplements, but not in vegans who obtain only 0–0.25 µg daily¹⁸. Low intake of animal-sourced foods may be involuntary owing to limited accessibility in the food supply, or voluntary owing to cultural, religious or personal restrictions. In addition, demands and needs vary depending on age and physiological status, such as pregnancy.

Although the Dietary Recommended Intakes for B12 have been defined for children and adolescents (by age), for adults, pregnant and breastfeeding women and elderly individuals aggregate figures and various sources have been used³⁸. In addition, the Dietary Recommended Intakes do not take factors that influence bioavailability into account, which seem to vary widely within these demographic groups, conditions of the gastrointestinal tract, overall total amount of B12 ingested and food sources^{1,3}. Bioavailability is in part related to the need to release B12 from its protein carriers in food. Bioavailability of B12 from milk is better than from other

Box 1 | Causes of vitamin B₁₂ deficiency

Conditions that cause vitamin B₁₂ (B12) deficiency and their associated pathogenetic bases:

- Pernicious anaemia: insufficient B12 absorption caused by a deficiency of intrinsic factor (usually owing to an autoimmune disease)
- Gastric disease or surgery (partial or complete gastrectomy or gastric reduction surgery): a deficiency of intrinsic factor
- Chronic atrophic gastritis (chronic inflammation causing loss of the gastric acid-producing cells) and an intake of drugs that affect gastric acid secretion or gastric pH (that is, proton pump inhibitors, histamine receptor 2 antagonists and antacids): B12 is not released from the food matrix owing to insufficient hydrochloric acid and low pepsin activity
- Pancreatic disease or pancreatectomy: B12 is not released from the haptocorrin complex owing to insufficient pancreatic enzyme activity
- Other intestinal diseases, ileal resection, parasitic infestations and bacterial overgrowth: impaired absorption of the B12–intrinsic factor complex
- Medications that affect B12 absorption or metabolism: reduction of serum B12 levels via known mechanisms (for example, cholestyramine) and unknown mechanisms (for example, metformin)
- Dietary factors such as general malnutrition, vegetarian or vegan diet, and chronic alcoholism: reduced B12 consumption
- Inherited disorders: decreased expression, binding activity or affinity of receptors and proteins involved in B12 trafficking and metabolism
- Miscellaneous: including HIV infection and nitrous oxide anaesthesia

Table 1 | Biomarkers of vitamin B₁₂ status

Biomarker; unit	Assay principle	Tentative reference interval*	Tentative cut-off value for B12 deficiency*	Tentative cut-off value for B12 repletion*	Major confounding factors
B12; pmol per litre	Protein-binding assay	200–600	<148	>221	Alterations in the plasma-binding proteins, haptocorrin or transcobalamin
Holotranscobalamin (transcobalamin-bound, active B12); pmol per litre	Immunological	40–100	<35	>40	Genetic variation in <i>TCN2</i> (REFS 73,209) and kidney function
Homocysteine; μmol per litre [‡]	Immunological, high-performance liquid chromatography or gas chromatography mass spectrometry	8–15	>15	<8	Folate and B6 deficiency, kidney and thyroid function, sex and age
Methylmalonic acid; μmol per litre	Liquid chromatography–mass spectrometry or gas chromatography mass spectrometry	0.04–0.37	>0.37	<0.27	Kidney function and <i>HIBCH</i> polymorphisms ¹¹⁷
4cB12 [§]	See formula below	–2.5–1.5	<–0.5	>0.5	Can be corrected for folate status and age

Blood tests are used to confirm a diagnosis of vitamin B₁₂ (B12) deficiency or to rule out the presence of poor B12 status. Most often, plasma B12 concentration — or as a more recent alternative holotranscobalamin — is used as the initial test. If the result is within the grey zone, analysis of the methylmalonic acid level is required. Homocysteine can replace methylmalonic acid in folate-repleted populations. *Reference intervals cover 95% of B12-replete individuals. Deficiency includes both clinical and subclinical deficiency. The exact values (except for 4cB12) for both reference intervals and cut-off limits may deviate in local settings³⁷. The values indicated are based on the cited literature and the experience of the authors, and define the cut-off value for subclinical B12 deficiency. [‡]In populations not receiving folic acid fortification. [§]Combined indicator of B12 status involving four parameters (4cB12) is a combination of B12, holotranscobalamin, homocysteine and methylmalonic acid, and is quantified as $\log_{10}((B12 \times \text{holotranscobalamin})/(\text{methylmalonic acid} \times \text{homocysteine}))$ test¹⁰⁶.

animal food sources³⁸. B12 from cow's milk may be more bioavailable than B12 from human breast milk, although this may relate, to some extent, to the higher concentration of B12 in cow's milk⁴⁴. Transcobalamin from cow's milk promotes intestinal cellular uptake of B12 *in vitro*⁴⁷. The possibility that transcobalamin or other factors present in cow's milk might enhance B12 absorption could have implications for both the prevention and the management of B12 deficiency.

Chemical inactivation of B12

The anaesthetic gas nitrous oxide chemically inactivates B12 through irreversible oxidation of its coenzyme form, methylcobalamin, at the active site of the B12-dependent methionine synthase reaction⁴⁸. Depending on the B12 status of the individual exposed to the gas, as well as the frequency and duration of its use, deficiency may be precipitous or gradual^{49,50}.

Absorption of B12

The main steps in B12 bioavailability, absorption, blood transport and intracellular metabolism are summarized in FIG. 4.

Pernicious anaemia. The virtually complete absence of intrinsic factor in patients with pernicious anaemia causes B12 deficiency through malabsorption of both dietary and recycled biliary B12, resulting in progressive exhaustion of B12 reserves in the body. Pernicious anaemia arises as a consequence of autoimmune gastritis, which is a chronic inflammatory disease of the fundus and body of the stomach, sparing the antrum. Initially asymptomatic and often associated with circulating parietal cell antibody directed against the gastric proton pump (gastric H⁺/K⁺ ATPase), the gastritis progresses over many years to type A chronic atrophic gastritis

with destruction of gastric parietal cells, which produce hydrochloric acid and intrinsic factor⁵¹. The gastritis may present initially as iron deficiency anaemia from loss of gastric acid, which is required for facilitating iron absorption⁵². Ultimately, loss of intrinsic factor together with production of neutralizing antibody against intrinsic factor leads to B12 malabsorption, megaloblastic anaemia (anaemia caused by defective DNA synthesis) and neurological complications, including neuropathy and subacute combined degeneration of the spinal cord. FIGURE 5 summarizes the key mechanistic pathways involved in pernicious anaemia. Mutations in *GIF*, encoding intrinsic factor, can also lead to an inherited form of B12 malabsorption and deficiency, which resembles pernicious anaemia, but without autoantibodies involvement².

Clustering of autoimmune gastritis with other organ-specific autoimmune diseases, including autoimmune thyroiditis and type 1 diabetes mellitus, suggests genetic predisposition to the development of gastritis. However, the precise predisposing genes have not been identified. A report of molecular mimicry by *H. pylori* antigens of proton pump antigens has raised the suggestion of a microbial trigger for the initiation of autoimmune gastritis⁵³, but this remains unproved. However, this observation raises important questions regarding the role of this ubiquitous microorganism in the causation of B12 malabsorption.

Impaired protein degradation. Digestion of food-derived proteins and haptocorrin derived from the saliva and bile (previously known as R binder) are essential for the transfer of B12 to intrinsic factor in the duodenum (FIG. 4). Haptocorrin is a B12-binding protein present in many body fluids. It protects B12 during its passage through the acidic environment in the stomach.

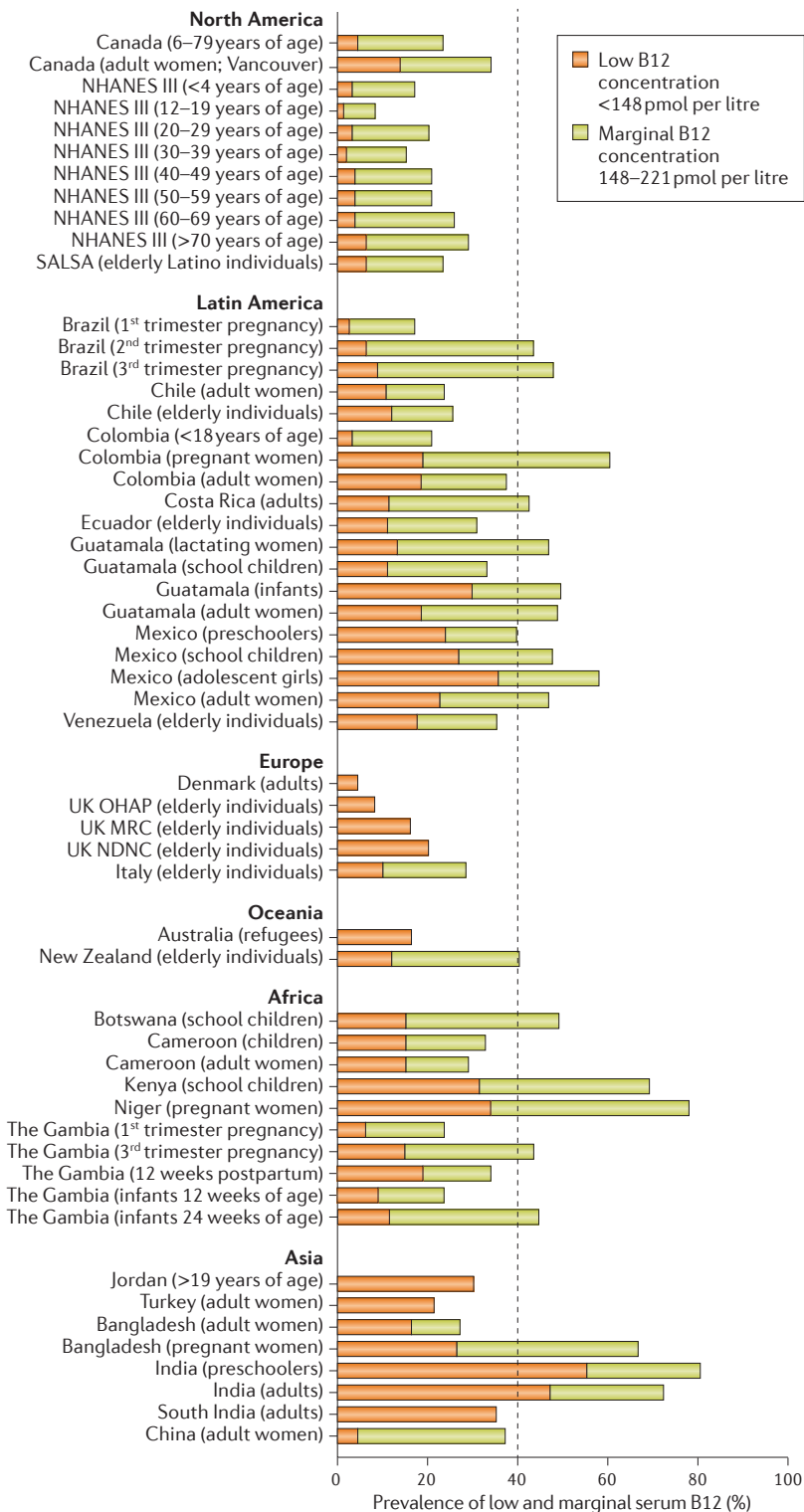


Figure 2 | Prevalence of low and marginal vitamin B₁₂. Global prevalence of low and marginal serum or plasma vitamin B₁₂ (B₁₂) concentrations from selected national surveys or large studies. Studies selected are nationally representative or large, with apparently healthy individuals categorized as having low or marginal serum or plasma B₁₂ concentrations defined as <148 pmol per litre and 148–221 pmol per litre, respectively. These figures are based on data extracted from three systematic reviews that focused on population-based studies assessing B₁₂ status^{33,195,196}, complemented with individual studies^{197–199} and ongoing studies. Data presented from Denmark, the United Kingdom, Turkey, Australia, South India and Jordan are limited to the prevalence of low B₁₂ status because marginal prevalence was not reported^{22,200–205}.

Reduced acid secretion in the stomach associated with chronic gastritis or long-term treatment with proton pump inhibitors impairs the release of dietary B₁₂ from food proteins and may produce deficiency, despite sufficient intrinsic factor secretion^{54–56}.

Conversely, both Zollinger–Ellison syndrome (caused by a gastrin-producing tumour and associated with gastric hyperacidity) and exocrine pancreatic insufficiency are rare causes of B₁₂ malabsorption due to low pH in the small intestine and impaired degradation of haptocorrin by pancreatic enzymes; both limit B₁₂ transfer from haptocorrin to intrinsic factor².

Bacterial overgrowth. Some microbial intestinal flora, such as *Pseudomonas* spp. and *Klebsiella* spp., are B₁₂ providers, others transform B₁₂ into other corrinoids (B₁₂ analogues), whereas the majority are B₁₂ consumers⁵⁷. This explains how B₁₂ can be a modulator of microbiota, whereas its bioavailability to the host can, in turn, be influenced by the microflora⁵⁷. The high specificity of intrinsic factor for B₁₂ limits the assimilation of analogues produced by the microflora⁵⁸. A genome-wide association study (GWAS) of B₁₂ deficiency in India identified single-nucleotide polymorphisms in genes encoding proteins involved in the glycan pathway, which could influence intestinal absorption and also influence the gut microbiota⁵⁹. Extreme conditions of bacterial overgrowth, as arise particularly in blind loop syndrome (resulting from surgical anastomoses that create gastrointestinal cul de sacs), produce a state akin to malabsorption of B₁₂ through extensive heterotrophic consumption of the nutrient. These syndromes may occur following gastrectomies, segmentary and ileocolic intestinal resection, inflammatory bowel diseases, diverticulosis as well as prolonged gastric achlorhydria (impaired gastric acid secretion). In addition, polymorphisms in *FUT2*, which encodes fucosyltransferase (an enzyme that catalyses fucose addition to form H-type antigens in exocrine secretions and red blood cell membranes⁶⁰), are involved in susceptibility to *H. pylori* infection. Fucosyltransferase can also influence the secretion of intrinsic factor, which is needed for the absorption of B₁₂ (REFS 59,61,62).

Impaired intestinal internalization. Two distinct mechanisms are involved in the intestinal uptake of B₁₂: specific and efficient uptake via receptor-mediated endocytosis of the intrinsic factor–B₁₂ complex in the terminal ileum mediated by a receptor complex known as cubam, which consists of the proteins cubilin and amnionless (encoded by *CUBN* and *AMN*, respectively)² (FIG. 4), and an inefficient, passive diffusion pathway that takes place throughout the intestine. Megalin (also known as low-density lipoprotein receptor-related protein 2 (LRP2)) — a multiligand transmembrane protein — can contribute to the internalization of B₁₂ in the intestine, in a manner that is analogous to its role in the reabsorption of transcobalamin in the kidney^{2,63}. However, patients with mutations that affect LRP2 have not been found to have any abnormality in B₁₂ absorption. Once internalized into the enterocyte, there is

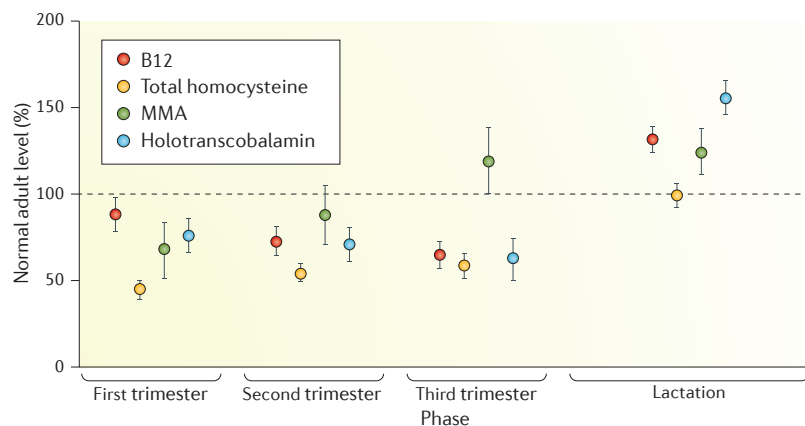


Figure 3 | Biomarkers of vitamin B₁₂ status during pregnancy and lactation. Levels of vitamin B₁₂ (B12) and associated markers presented as percentage of normal levels based on a population of 207 non-pregnant, non-lactating women 18–40 years of age with serum B12 levels of 225–750 pmol per litre, serum folate levels of >10 nmol per litre and normal renal function (a glomerular filtration rate of >75). Percentage change during pregnancy and lactation was calculated using the following median levels: B12: 359 pmol per litre, homocysteine: 9.3 μmol per litre and methylmalonic acid (MMA): 0.12 μmol per litre. Data during pregnancy for B12 and total homocysteine levels are based on REF. 29; data on MMA levels are based on REF. 28; and data on B12 status from the lactational period are based on REF. 43. Percentage change of holotranscobalamin levels during pregnancy is based on REF. 28 and during lactation on REF. 31. Errors bars represent the 10th and 90th percentiles from the geometric mean.

some evidence that multidrug resistance protein 1 may be involved in the process to export the vitamin into the bloodstream⁶⁴. B12 is exported into the bloodstream where it binds to transcobalamin for delivery of the vitamin to all cells in the body.

Imerslund–Gräsbeck syndrome (megaloblastic anaemia 1; Online Mendelian Inheritance in Man (OMIM) #261100) is a rare recessive disorder caused by mutations in *CUBN* or *AMN* that result in intestinal B12 malabsorption, anaemia and deficient renal protein reabsorption^{65,66}. Various phenotypes exist, including decreased binding activity or affinity by the receptor or expression of an unstable receptor⁶⁷.

Inflammatory bowel diseases, infection and drugs. Coeliac disease or Crohn's disease affecting the ileum results in B12 malabsorption through villous atrophy and mucosal injury. Two parasitic infestations produced by a fish tapeworm, *Diphyllobothrium latum*, and the protozoan *Giardia lamblia* can lead to malabsorption through B12 trapping by the parasite. Tropical sprue results in malabsorption through intestinal villous atrophy. B12 malabsorption may also be due to medical interventions. Cholestyramine (a bile acid resin used to treat hypercholesterolaemia) can chelate intrinsic factor, whereas colchicine (used for acute gout) and several antibiotics (including the anti-tuberculosis drug para-aminosalicylic acid) can act as inhibitors of intrinsic factor–B12 endocytosis. The duration or frequency of use of these drugs is usually insufficient to result in clinical B12 deficiency, in contrast to the long-term use of drugs such as proton pump inhibitors, histamine H2 receptor antagonists and metformin, as noted above^{1,5}.

Disrupted transport and intracellular metabolism

About 20% of total plasma B12 levels are bound to transcobalamin and are, therefore, available to the cells via receptor-mediated uptake. The major, remaining, fraction is bound to a circulating form of haptocorrin. The transcobalamin-bound B12 fraction (holotranscobalamin) is internalized by receptor-mediated endocytosis via the receptor CD320 antigen (in the brain and other tissues) or LRP2 (in the luminal portion of the kidney tubule) and is subsequently degraded in lysosomes⁶⁸. Intracellularly, B12 is reduced and converted into its coenzymatically active forms for use in two intracellular processes: the cytoplasmic conversion of homocysteine into methionine and the mitochondrial metabolism of methylmalonyl-CoA to succinyl-CoA. There are several inherited disorders that affect the sequential steps in the assimilation, transport and intracellular processing of B12 (REFS 69,70) (FIG. 4b). The crucial role of transcobalamin in B12 transport and delivery is exemplified by clinical reports that >20 inherited mutations in *TCN2* (encoding transcobalamin 2) manifest as failure to thrive and severe disease in infancy. These mutations are associated with severe megaloblastic anaemia and neurological problems with fatal outcome if not recognized and promptly treated with high doses of B12 (REFS 70–72). Other *TCN2* polymorphisms have less-profound effects on B12 metabolism, including possible differences in the expression level and blood concentration of transcobalamin and indices of B12 status in healthy individuals^{73,74}.

The inherited disorders of intracellular B12 metabolism are classified as complementation groups cblA–J by complementation phenotyping of fibroblasts^{70,75} (FIG. 4b). They encompass several genes, which, respectively, encode or regulate the transcription of proteins involved in the intracellular trafficking and metabolic activation of B12. Mutations that block the lysosomal release of B12 or the cytoplasmic function of methylmalonic aciduria and homocystinuria type C protein (MMACHC) lead to impaired synthesis of adenosyl-B12 and methyl-B12 with consequent increase in the levels of homocysteine and MMA. These disorders are characterized by megaloblastic anaemia, neurological involvement, stunted growth and sometimes retinopathy. Mutations that block only the synthesis of adenosyl-B12 result in increased levels of MMA and produce acute encephalopathy with ketoacidosis, hyperammonaemia and hyperglycaemia. Mutations that block the synthesis of methyl-B12 increase only the levels of homocysteine and are manifested by megaloblastic anaemia and encephalopathy during the first few months of life.

Complications of B12 deficiency

Cellular and molecular consequences. The common consequence of B12 deficiency and genetic disorders affecting B12 metabolism is a cellular deficit in one or both of the coenzyme forms of B12 (that is, adenosyl-B12 and methyl-B12). At the molecular level, B12 deficiency leads to an impaired methylation and impaired metabolism of methylmalonate, which is derived from the catabolism of certain amino acids and fatty acids (FIG. 1). Methyl-B12 deficiency results in homocysteine

Box 2 | Vitamin B₁₂ in breast milk

Vitamin B₁₂ (B12) levels in breast milk are highly correlated with maternal serum B12 levels^{31,178}. Although maternal B12 levels tend to increase postpartum, the level of increase depends on pre-pregnancy stores, B12 intake (diet and supplements) and depletion of the stores during pregnancy. Reported B12 concentrations in human milk vary substantially from 150 to 700 pmol per litre^{41,179}. Breast milk concentrations in American women are reported to be 300 pmol per litre³¹ to >600 pmol per litre¹⁸⁰, whereas the levels in India and Guatemala are very low (<100 pmol per litre)^{146,178} or even undetectable in those with very low dietary B12 intake. Levels in human milk fall progressively during the lactation period⁴¹. The estimated B12 intake of the breastfed infant is maximal at 12 weeks and is reduced by 50% at week 24 (REF. 179). Most commercially prepared infant formulas are enriched with B12 up to concentrations of 800–1,200 pmol per litre^{179,181}, and better B12 status (higher levels of B12 and lower levels of homocysteine and methylmalonic acid) is reported in formula-fed than in breastfed infants^{40,41,44}.

accumulation and reduced synthesis of methionine and S-adenosylmethionine. Homocysteine accumulation can induce cellular stress, apoptosis and homocysteinylolation of functional proteins in the blood and tissues (the formation of covalent adducts with ε-amino group of their lysine residues)⁷⁶. S-adenosylmethionine is the methyl donor that is required for epigenetic reactions, including methylation of DNA, histones and other regulators of gene expression. Adenosyl-B12 deficiency leads to an accumulation of MMA, the consequences of which are not clear, as discussed below.

Recent data also indicate a strong link between B12 deficiency and cellular stress through the reduced expression of NAD⁺-dependent protein deacetylase sirtuin 1 (hSIRT1), the subsequent increased acetylation of heat shock factor 1 (HSF1) and the impaired expression of heat shock proteins⁷⁷. Consistently, fibroblasts from patients with cblC, caused by mutations in *MMACHC* (FIG. 4b), showed expression changes in genes encoding key proteins of cellular endoplasmic reticulum that were involved in oxidative stress (such as heat shock proteins, ubiquitins and proteins involved in the glutathione pathway)⁷⁸. The increased production of reactive oxygen species (ROS) triggers endoplasmic reticulum stress and apoptosis⁷⁹.

The causes of the neurological complications remain to be determined. The decreased synthesis of succinyl-CoA and the accumulation of MMA could, theoretically, contribute to the neurological manifestations of B12 deficiency through the formation of odd chain and methyl-branched chain fatty acids⁴⁹, but evidence to support this theory is scant. Studies of inherited defects have led to the conclusion that a lack of methyl-B12 or methionine synthase inhibition is the major cause of the neurological lesions in B12 deficiency. In addition, the neurological complications might be due to inflammation^{80,81}, oxidative stress⁸² and microvascular disease associated with hyperhomocysteinaemia⁸³.

Low B12 status and increased levels of homocysteine are also associated with reduced methylation of the promoters of the genes encoding amyloid precursor protein and γ-secretases, leading to increased amyloid-β production. S-adenosylmethionine administration reverses these effects and improves spatial memory performance⁸⁴.

B12 deficiency induces an increased expression of protein phosphatase 2A, nerve growth factor and tumour necrosis factor, and decreased expression of epidermal growth factor in cell and animal models^{85,86}. These changes are consistent with an influence of B12 deficiency on myelin homeostasis and on the amyloid and tau pathways of neurodegenerative disorders.

Neurological manifestations. B12 deficiency affects the nervous system, resulting in demyelination of peripheral and central neurons¹⁴, which is generally considered to be the mechanism underlying the classic myeloneuropathy of B12 deficiency. The long tracts of white matter in the posterior and lateral columns of the spinal cord containing sensory neurons that are responsible for the conduction of vibration and position are particularly susceptible to demyelination, but motor neuron myelination can also be affected. The neurological manifestations of B12 deficiency can precede the appearance of haematological changes and may even occur in the absence of any haematological complications⁶.

Adequate B12 status is crucial for normal neurodevelopment, as demonstrated by the clinical picture presented in children with inherited disorders of B12 metabolism⁸⁷. The first postnatal months are the most dynamic and vulnerable period of brain development. The signs and symptoms of B12 deficiency in childhood depend on the age of the child and on the severity and duration of the deficit⁸⁸ (BOX 3). In addition, a randomized, double-blind B12 intervention study provided evidence of functional motor impairment in infants with a biochemical profile that was indicative of moderate B12 deficiency⁴⁵. A single intramuscular dose of 400 μg of hydroxy-B12 resulted in biochemical evidence of B12 repletion and improvement in motor function and regurgitations, suggesting that adequate B12 status is important for the rapidly developing nervous system⁴⁵. Although B12 treatment in deficient infants and toddlers commonly causes rapid progress in motor development and improvement in clinical symptoms^{45,89}, prolonged deficiency may result in permanent developmental disabilities, even after optimal treatment⁸⁹.

As with folate deficiency⁹⁰, maternal low B12 status and B12 deficiency during pregnancy and lactation can have consequences for the offspring (BOX 3), including neural tube defects^{91,92}. Although there is little clarity on the consequences of subclinical low B12 status in adults, there is evidence that, if allowed to persist, it may increase the risk of initiation or rate of progression of several chronic diseases associated with ageing (BOX 3).

Low B12 status has been shown to be the primary modifiable cause of increased plasma levels of homocysteine in a folic-acid-fortified population residing in a high-income country⁹³. This is particularly important for older adults in whom increased levels of homocysteine associated with low B12 status predict accelerated brain atrophy and incident clinical cognitive impairment^{94–97}. Analysis of NHANES data has shown a higher risk of anaemia and cognitive impairment in older adults who have low serum levels of B12 and increased serum levels of folate compared with those with low B12 but without increased

folate levels⁹⁸. In addition, folic acid supplementation in patients who are B12 deficient partially or temporarily restores haematological complications that arise from impaired DNA synthesis^{1,4,5}, whereas neurological complications are unaffected or aggravated^{98,99}.

Haematological manifestations. The haematological effect of B12 deficiency is megaloblastic anaemia, which results from disruption of DNA synthesis. When B12 is deficient, H₄-folate synthesis is impaired (FIG. 1), which limits the supply of the required form of folate for the synthesis of thymidylate and DNA. This causes misincorporation of dUTP in place of thymidine triphosphate during

DNA synthesis^{1,4,5}. DNA synthesis in tissues undergoing rapid cellular turnover, such as the haematopoietic system, is particularly affected. Unbalanced growth in dividing bone marrow cells produces abnormally large cells with fine, immature-looking nuclear chromatin. This predominantly affects erythroid precursors, giving rise to anaemia with abnormally large red cells (macrocytes). Other haematopoietic cells are also affected, resulting in gigantic granulocyte precursors in the marrow and neutrophils with more than the normal number of nuclear lobes (hypersegmented neutrophils) in the blood (FIG. 6). In addition to anaemia, there may also be a decrease in the numbers of all blood cells (pancytopenia).

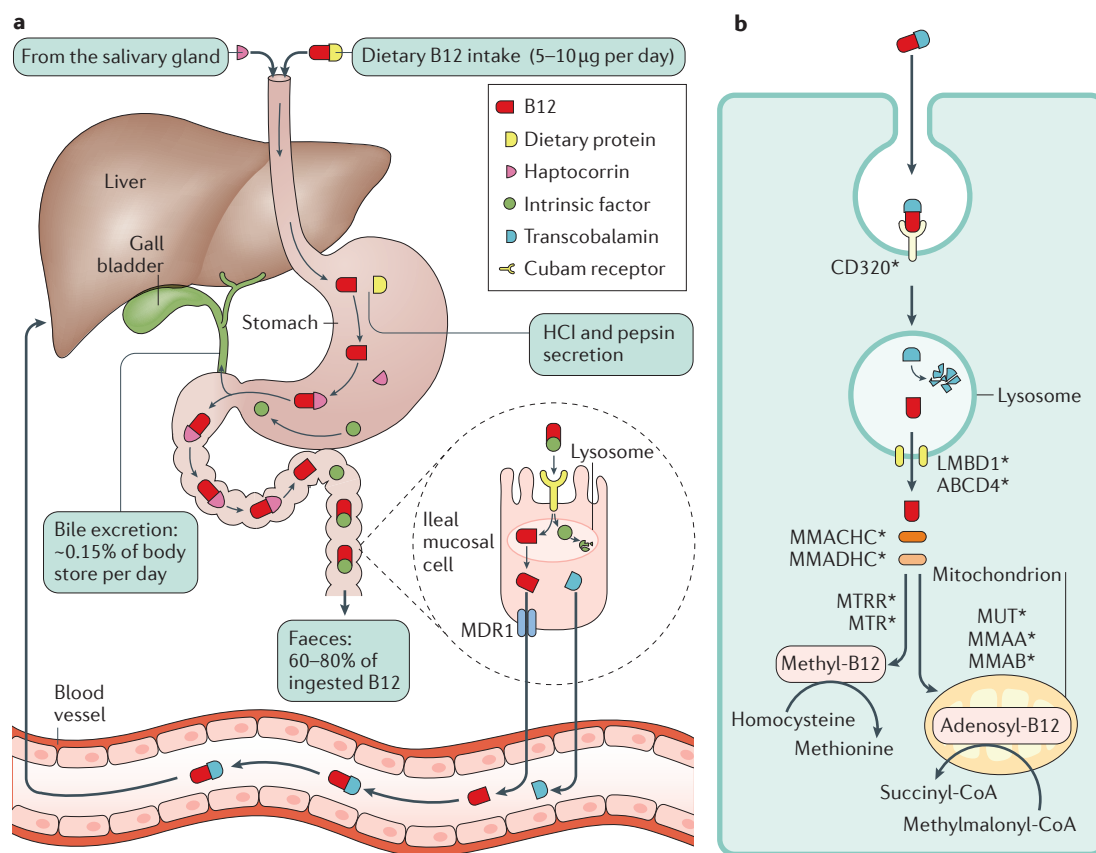


Figure 4 | Absorption, enterohepatic circulation and intracellular metabolism of vitamin B₁₂. **a** | Vitamin B₁₂ (B12) is mainly derived from animal sources. Following intake, it is released from its food carrier proteins by proteolysis in the acidic environment of the stomach, where it binds to haptocorrin⁵⁸. Haptocorrin is produced by the salivary glands and protects B12 from acid degradation. Degradation of haptocorrin and the pH change in the duodenum favour B12 binding to gastric intrinsic factor, which is produced by gastric parietal cells. The intrinsic factor–B12 complex binds to the cubam receptor (consisting of cubilin and amnionless^{2,65,66}). This receptor mediates the uptake of the intrinsic factor–B12 complex in the enterocytes of the distal ileum via receptor-mediated endocytosis. After lysosomal release, B12 exits via the basolateral membrane of the enterocyte, facilitated by multidrug resistance protein 1 (MDR1), and binds to transcobalamin, the blood carrier of B12 that is responsible for cellular delivery of B12 (REFS 2,58,64). The majority of B12 is stored in the liver; some B12 is excreted in bile and undergoes enterohepatic circulation^{167,206}. **b** | Cellular uptake of B12 involves the CD320 receptor present on all cell types. Studies on CD320-knockout mice suggest that other yet to be identified receptors for transcobalamin may exist²⁰⁷. Several inherited disorders — designated CblA to CblJ — are associated with mutations in genes encoding proteins involved in intracellular B12 metabolism. These include (indicated with *): two lysosomal proteins (lysosomal cobalamin transporter (LMBD1) and the lysosomal membrane transporter ABCD4); the cytoplasmic chaperones, methylmalonic aciduria and homocystinuria type C protein (MMACHC) and MMADHC, whose function has not been established; the mitochondrial enzyme methylmalonyl-CoA (MUT) as well as two mitochondrial proteins (cblA and cblB) involved in the transfer and maintenance of the adenosyl group to adenosylcobalamin, mutations of which result in methylmalonic aciduria cblA and cblB type (MMAA and MMAB), respectively; and the two respective target enzymes of methyl-B12 (methionine synthase reductase (MTRR) and methionine synthase (MTR))⁶⁹. HCl, hydrochloric acid.

Diagnosis, screening and prevention

Diagnosis

The clinical manifestations of B12 deficiency are varied and may mimic or be mimicked by various other diseases. Indeed, the clinical presentation of severe megaloblastic anaemia can closely mimic haemolytic anaemia, thrombotic thrombocytopenic purpura and myelodysplastic syndromes^{100–103}. Delay in diagnosing B12 deficiency may be life-threatening, and inappropriate treatment of the mimicker entities in a patient with underlying B12 deficiency is a major clinical error. Making a specific diagnosis of deficiency is paramount. Before committing a patient to lifetime B12 treatment, the deficiency should be documented by pre-treatment increases in the levels of MMA or homocysteine, further underscored by the presence of anti-intrinsic factor antibodies or a history of the relevant post-surgical states that could lead to B12 deficiency, such as gastrectomy, gastric reduction or ileal resection. Empirically, treatment with high-dose B12 while awaiting the results of more specific testing does not cause harm.

Who to test? Two groups of patients should be considered for diagnostic testing for B12 deficiency. The first group comprises those with clinical evidence of B12 deficiency, including macrocytic anaemia and/or neurological symptoms. In such patients, laboratory tests may help to confirm the diagnosis, but a firm clinical suspicion of B12 deficiency warrants immediate treatment with B12 (REF. 104).

The other, much larger group of patients who experience nonspecific symptoms, quite often not including anaemia, presents a greater challenge. This group includes elderly individuals, individuals on a diet with limited amounts of B12, such as vegans, individuals with impaired fertility, patients with gastrointestinal diseases, and patients with non-characteristic and unexplained haematological or neurological symptoms⁴. Within this group, individuals with poor B12 status are mainly identified by biomarker measurements (TABLE 1). However, this approach does have its limitations. Taken individually, serum B12 or holotranscobalamin values

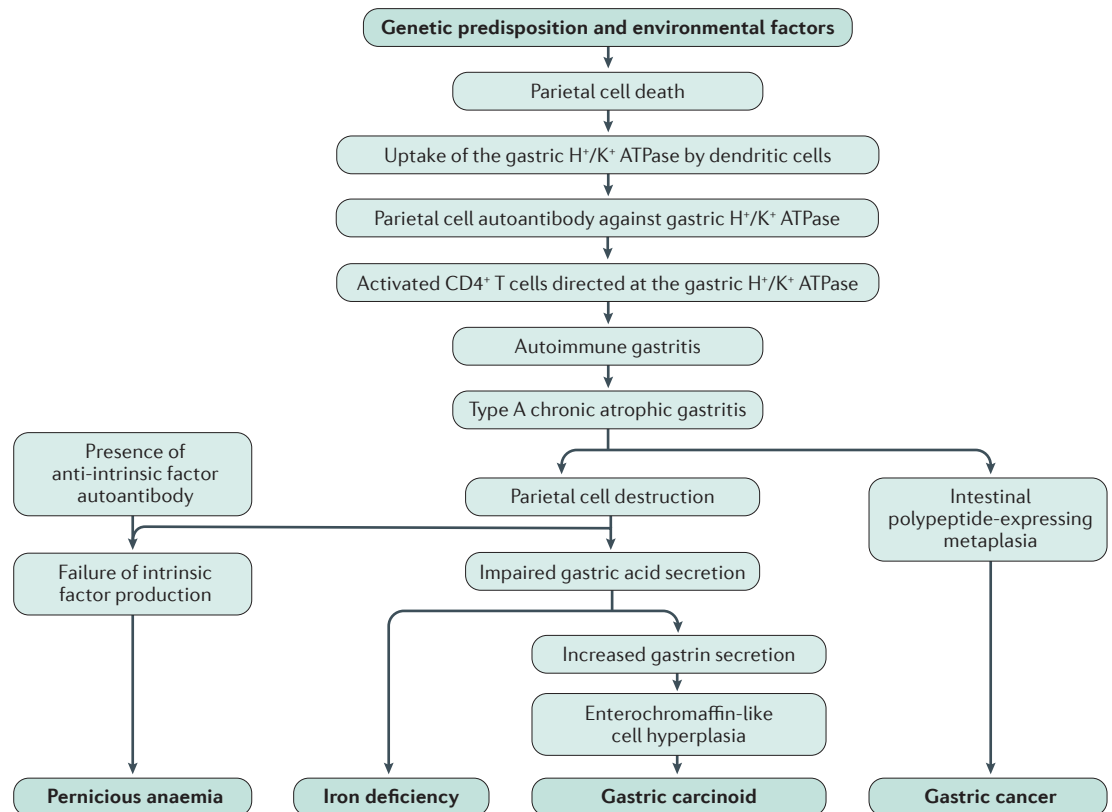


Figure 5 | Mechanism and complications of autoimmune gastritis. Autoimmune gastritis and type A chronic atrophic gastritis can develop in genetically predisposed individuals. Environmental risk factors (such as *Helicobacter pylori* infection) might also have a role. Apoptosis of parietal cells might result in the release of gastric H⁺/K⁺ ATPase constituents, which are taken up by gastric dendritic cells that then migrate to the draining gastric lymph nodes to activate naive CD4⁺ T cells. The gastric H⁺/K⁺ ATPase-activated CD4⁺ T cells then migrate to the gastric mucosa to initiate tissue damage by binding to MHC class II molecules and by activating FAS-dependent mechanisms²⁰⁸. Gastritis is marked by the presence of autoantibodies targeting the gastric H⁺/K⁺ ATPase. Chronic atrophic gastritis also represents a risk factor for gastric cancer arising from polypeptide-expressing intestinal metaplasia, as well as enterochromaffin-like cell hyperplasia arising from gastrin hypersecretion by antral G cells predisposing to gastric carcinoid. Pernicious anaemia arises from intrinsic factor deficiency as a result of loss of intrinsic factor-producing gastric parietal cells and the presence of intrinsic factor autoantibodies. Loss of parietal cells that also produce acid via the gastric H⁺/K⁺ ATPase can lead to iron deficiency anaemia.

Box 3 | Consequences and symptoms of vitamin B₁₂ deficiency or low vitamin B₁₂ status***Symptoms of B12 deficiency in infants**

- Feeding difficulties⁸⁸
- Regurgitations⁸⁸
- Constipation⁸⁸
- Apathy⁸⁸
- Irritability⁸⁸
- Twitching, tremors and myoclonic jerks⁸⁸
- Slow growth, small head circumference and brain lesions
- Developmental delay, including reduced gross motor development, smiling and babbling⁸⁸
- Pancytopenia

Consequences of low maternal B12 status or deficiency in infants

- Neural tube defects reported in infants of mothers with low B12 status in folic acid-fortified and non-fortified populations^{91,92}
- Effects on infant development, including stunting, cerebral atrophy, hypotonia, lethargy, developmental delays and abnormal electroencephalogram⁸⁸
- Contribution to adult cardiovascular disease, neurodegenerative disorders and psychiatric illness through increased levels of circulating homocysteine¹
- Contribution to the development of diabetes mellitus through effects on insulin and lipid metabolism³⁷

Symptoms of B12 deficiency in older children

- Lower school performance
- Reduced weight¹⁸²
- Reduced height and head circumference¹⁸²
- Macrocytic anaemia¹⁸²
- Normal or increased weight^{183,184}
- Normal haematological values¹⁸⁴
- Impaired mental and social development, short-term memory and attention^{185–187}

Consequences of low B12 status or deficiency in adults

- Megaloblastic anaemia or macrocytic anaemia^{1,4,5,123}
- Subacute combined degeneration of the spinal cord^{4,6,168}
- Impaired sensory and peripheral nerve function¹⁸⁸
- Cognitive impairment^{127,130,189,190}
- Depression¹²⁶
- Bone disease^{135,191}
- Hearing loss¹⁹²
- Macular degeneration¹⁹³

The severity of vitamin B₁₂ (B12) deficiency varies and is defined in the source references. *The data are derived from several sources as indicated by the references.

below the lower level of the reference interval have low specificity for identifying true vitamin deficiency. Poor sensitivity of these tests also means that true B12 deficiency may often go unrecognized^{9,105}. In general, if unequivocal or profound B12 deficiency is present, it is important that it be diagnosed and treated as swiftly as possible. Indeed, once B12-related neurological damage has occurred, it might not completely reverse following treatment.

Diagnostic tests and biomarkers. Despite the limitations, biomarkers — total B12, holotranscobalamin, MMA and homocysteine, as well as combinations of these measurements — are increasingly used¹⁰⁶. Assay characteristics and confounding factors are summarized in TABLE 1.

Serum B12 level is usually the first-line test. In general, a value well below the lower limit of the reference interval is indicative of probable B12 deficiency, whereas a value well above this limit indicates a sufficient B12 status. Important exceptions may occur in the presence of circulating antibodies against intrinsic factor in patients with pernicious anaemia, in whom spuriously normal B12 levels have been reported¹⁰⁷. In daily practice, the same reference interval is used across age and sex, even though the reference interval broadens with age¹⁰⁸. The reference interval is method dependent and, therefore, no universal cut-off limits can be stated. The level of B12 is influenced by the concentration of the two plasma-binding proteins. High levels of haptocorrin are associated with liver diseases, myeloproliferative diseases, such as chronic myeloid leukaemia, and other malignancies, explaining why an increased level of B12 may be encountered in these conditions¹⁰⁹.

Active, transcobalamin-bound B12 (holotranscobalamin) should, theoretically, be a more-sensitive marker for B12 deficiency than total serum B12 levels¹¹⁰. Measurement of holotranscobalamin is gradually being incorporated into the clinical laboratory, but so far, the biomarker has proved only marginally better than total B12 levels^{111–113}.

Homocysteine and MMA accumulations in plasma marks B12 deficiency⁴, but homocysteine levels are also influenced by other parameters (TABLE 1). The method of blood collection and plasma separation also presents some pre-analytical challenges for homocysteine measurements¹¹⁴. MMA level is the most specific and sensitive single marker for B12 deficiency, and is often used as the ‘gold standard’ for defining B12 status^{115,116}. The major drawbacks are availability and cost of the assay. A major advantage is that the analyte is very well standardized, which allows for a uniform interval of reference of ≤ 0.27 μmol per litre, and a cut-off point that indicates poor B12 status of >0.37 μmol per litre^{106,115}. However, genetic determinants of MMA cut-offs have recently been challenged in a GWAS of genetic factors that influence plasma MMA levels¹¹⁷. *HIBCH*, which encodes a protein involved in valine catabolism, was found to contain a single-nucleotide polymorphism (rs291466) that was the most common genetic driver of plasma MMA levels. This common polymorphism is widespread in populations (with a minor allele frequency of 0.43), and people who are homozygous for the common allele have plasma MMA concentrations that are approximately 46% higher than those with the rarer allele¹¹⁷. This B12-independent determinant of plasma MMA may influence its usefulness as a B12 status indicator, when used as a single determinant.

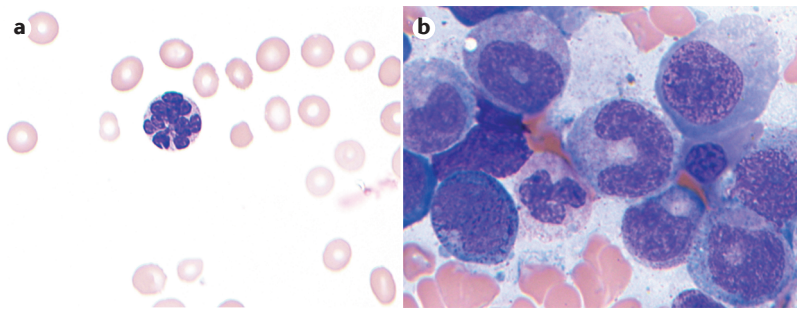


Figure 6 | Blood and bone marrow morphological changes in vitamin B₁₂ deficiency. **a** | Blood smear showing nuclear hypersegmentation (more than five lobes) of a neutrophil with several larger than normal oval erythrocytes present. **b** | Bone marrow aspirate smear showing abnormally large erythroid precursor cells with fine, immature-looking nuclear chromatin and large 'giant' granulocytic band form with horseshoe-shaped nucleus. Nuclear maturation is retarded in both the erythroid and the granulocytic precursors owing to faulty DNA synthesis.

This is mainly relevant in older adults who have higher levels of MMA that are unrelated to B12 status than younger individuals. Elderly individuals who have the genotype that leads to higher levels of MMA could easily have MMA concentrations of >0.37 μmol per litre, without being deficient in B12. The relevance of this polymorphism in the clinical setting warrants further study.

Many laboratories use a diagnostic strategy that involves more than one biomarker, most often using B12 levels as the initial test and MMA or homocysteine levels as the second-line test¹¹⁸. Recently, this approach has been further improved by the development of an equation that includes two to four biomarkers. This newly introduced B12 index is termed cB12 (combined indicator of B12 status)¹⁰⁶. The advantage of cB12 is that it is independent of local reference intervals and can be adjusted to correct for folate status and age. This index shows a stronger association with haemoglobin concentrations, cognitive function and peripheral nerve conductivity than single markers. At this time, the prospective clinical usefulness of the index remains to be confirmed.

Diagnosing B12 deficiency in elderly individuals. B12 deficiency is particularly difficult to identify in elderly individuals because the typical haematological and neurological manifestations of clinical B12 deficiency are frequently not present or are easily confused with similar manifestations of other common diseases of older age, most notably dementia. Moreover, the sensitivity of the biomarkers MMA and plasma homocysteine lessens with older age because of impaired kidney function^{8,119–121}. Haematological symptoms occur in $<50\%$ of individuals, and, even when present, macrocytic anaemia in older individuals is frequently due to other haematological disorders, such as myelodysplasia, or the use of medications that have no bearing on B12 status^{122,123}. As noted above, neuropsychiatric and neurological symptoms are more-frequently observed in elderly individuals who are B12 deficient^{6,124–130}, but these symptoms generally tend to be nonspecific and

similar to the symptoms encountered in many other chronic diseases of ageing. As a result, management relies heavily on clinical judgement. Several intervention studies have focused on the effect of correcting hyperhomocysteinaemia with B12 supplementation. Some, but not all, studies have demonstrated improvements in disease state^{95,131–136}. In one study, supplementation with homocysteine-lowering vitamins that included B12 was effective in reducing the rate of brain atrophy among patients with increased levels of homocysteine at baseline^{94,96}.

Diagnosing B12 deficiency in infants. As stated above, B12 deficiency caused by inherited defects may occur early in life. Typically, infants present with pancytopenia and failure to thrive. In addition, nutritional B12 deficiency should be considered in young, mainly breastfed infants with feeding difficulties, subtle neurological symptoms and delayed motor development, particularly if the B12 intake of the mother has been low. Owing to the subtle symptoms of B12 deficiency and the large variations in normal development during infancy (BOX 3), B12 deficiency is reported to have a median diagnostic delay of 4 months in this age group⁸⁸. A plasma homocysteine level of ≥ 6.5 μmol per litre has been suggested as a cut-off level for defining B12 deficiency in infants⁴⁵. This represents the 97.5 percentile in 4-month-old infants who are given a single intramuscular dose of 400 μg of hydroxy-B12 at 6 weeks, to render them B12 replete⁴³.

Determining the cause of B12 deficiency. In terms of optimal patient management, establishing a cause of B12 deficiency is highly desirable. To achieve this, a key step is to determine whether the cause is low intake or malabsorption, and, if the latter, whether the defect is gastric or intestinal. Over the past two decades, use of the gold-standard test for assessing B12 absorptive capacity — known as the Schilling test — has dwindled to essentially zero owing to the lack of availability of radiolabelled B12 that is required for the test and high cost, among other factors. No substitute test has been validated for widespread clinical use. The CobaSorb test, which is based on an increment in the serum holotranscobalamin level following oral dosing with B12, shows promise in that no increase in the level of holotranscobalamin occurs in patients who lack intrinsic factor as well as in patients with Imerslund–Gräsbeck syndrome^{137,138}, but the test has the limitation that it cannot be used once the patient is treated with B12 (REF. 139). A ¹⁴C-labelled form of B12 produced through microbial synthesis offers the possibility of being used to measure B12 absorption, but this has not yet been developed into a clinical test¹⁴⁰.

In the absence of a robust and reliable absorption test, other approaches can be used. To diagnose classic pernicious anaemia¹⁴¹, the detection of autoantibodies against intrinsic factor and gastric H⁺/K⁺ ATPase can be used, as well as the levels of pepsinogen or gastrin¹⁴². Although a negative result for anti-intrinsic factor autoantibodies does not exclude pernicious anaemia, a positive result

has 100% specificity. However, a positive result for anti-H⁺/K⁺ ATPase autoantibodies is nonspecific as it may be seen also in patients without pernicious anaemia¹⁴³. The finding of atrophic gastritis during upper endoscopy may provide an important lead for suspecting pernicious anaemia, as all patients with pernicious anaemia have atrophic gastritis; when atrophic gastritis is found, other non-immune causes of atrophic gastritis should be excluded.

Curiously, a decline in MMA levels following B12 intake or injection has so far not been developed into a standardized functional test, although this parameter may prove very valuable for confirming the presence of B12 deficiency and also for adjusting treatment.

When B12 deficiency is found or suspected in the paediatric age group, inborn errors of metabolism affecting cobalamin processing should be considered in the differential diagnosis, and suitable tests, either through complementation phenotyping or genetic analysis, should be carried out^{69–71,75}.

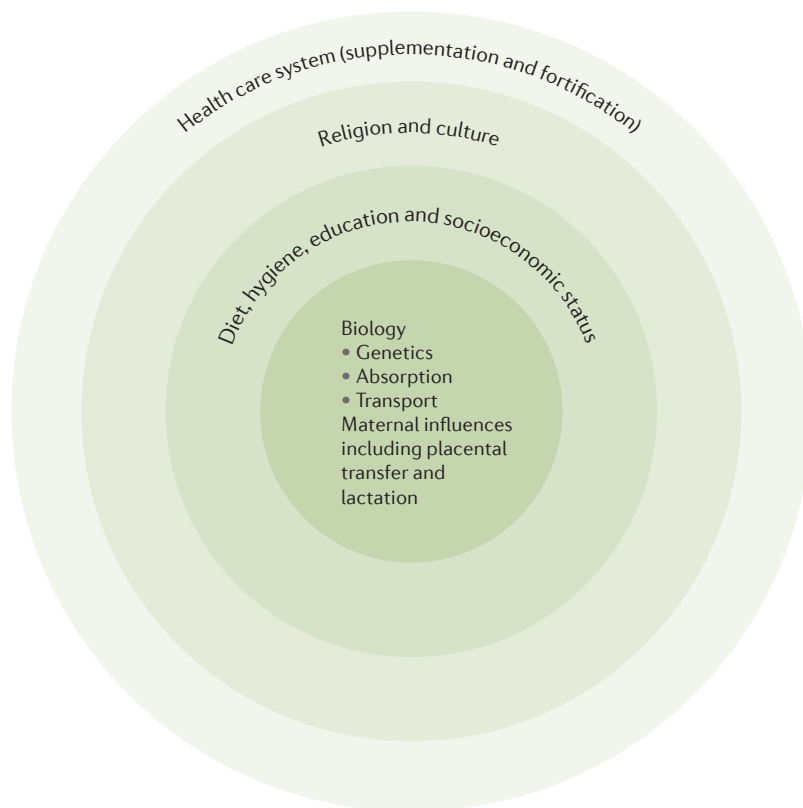


Figure 7 | Determinants of vitamin B₁₂ status. Important factors that affect the demand and supply of vitamin B₁₂ (B12) on the individual and population levels and throughout the patient's lifetime are highlighted. It is striking that the mother exerts a triple influence on the B12 status of the offspring: genetics, intrauterine and lactational B12 transfer, and postnatal family environment (socioeconomic status, hygiene, diet, religion and culture). The father exerts a double influence: genetic and family environment. A female child will continue to propagate the direct maternal influences into the next generation. The suspected role of epigenetics in all three processes awaits elucidation. The nutritional status of the population and, therefore, the public health measures to improve it depend on the socioeconomic factors, religious and cultural practices, and the public health policies of the local, national and international regulatory systems. Although targeting the B12 deficiency on an individual level is needed, more widespread interventions targeting the population are needed to prevent B12 deficiency.

Prevention

Pregnant women, infants and children. Deficiency of B12 is emerging as a public health concern in many low-income countries. A WHO Consultation identified infants, preschool children and pregnant and lactating women as the most vulnerable groups¹⁴⁴. The strategies for prevention of B12 deficiency and its public health significance may be discussed in a life-course model¹⁴⁵. FIGURE 7 summarizes the factors influencing B12 status across the life course and stresses the importance of direct transfer of B12 from the mother to the baby during pregnancy and lactation. Maternal B12 status during pregnancy and cord blood B12 concentration predict B12 status of the offspring well into early adulthood, highlighting a crucial role for maternal vitamin status to prevent B12 deficiency in the next generation. Importantly, this transgenerational cycle becomes self-perpetuating if the fetus is female. This suggests that improving the nutrition of young girls has the potential of improving the 'legacy' B12 status of the population for many generations and reducing associated morbidity. This novel idea could have a substantial long-term benefit in public health. Maternal B12 status is negatively affected by a combination of diets poor in animal-sourced foods, high fertility rates and short inter-pregnancy intervals, and further aggravated by sociocultural factors such as early marriage with adolescent pregnancies, as well as dietary taboos during pregnancy and lactation.

Oral supplementation of urban Indian women with B12 (50 µg daily) throughout pregnancy and early lactation significantly increased the B12 status of mothers (median: 184 versus 105 pmol per litre; $P < 0.001$) and infants (199 versus 139 pmol per litre; $P = 0.01$) compared with non-supplemented controls. Supplementation improved median breast milk B12 concentration 6 weeks post-partum (136 versus 87 pmol per litre; $P < 0.001$) and was also associated with a drop in the incidence of intrauterine growth retardation¹⁴⁶. In Bangladesh, maternal supplementation with 250 µg of B12 daily during most of the pregnancy and 3 months of lactation resulted in improved maternal and infant status, substantially increased B12 in colostrum and breast milk, and improved influenza H1N1 vaccine-specific response in the mothers¹⁴⁷.

General prevention strategies. The overall long-term strategy for controlling B12 deficiency is to promote consumption of foods rich in B12. Vegetarians will always have difficulty achieving this because the natural dietary source of B12 is animal-origin foods, unless they consume eggs, milk and other dairy products, or foods fortified with the vitamin. In addition to ethics, religion and culture, socioeconomic factors may limit the intake of animal-origin foods (FIG. 7).

B12-containing plant-derived food sources consumed during pregnancy include green and purple lavers (Nori) and blue-green algae or cyanobacteria (Spirulina). Nori is considered suitable for humans, but it is not widely available, whereas the more widely available Spirulina may contain pseudo-B12, a biologically

inactive analogue¹⁴⁸, which would not be beneficial and might even be harmful. Thus, dairy products and eggs remain the only acceptable animal source of B12 for vegetarians. A study from Pune reported improvement in B12 status in young, healthy, B12-deficient vegetarians with regular intake of non-fortified milk (600 ml per day)¹⁴⁹, whereas a food-based micronutrient-rich snack (dried fruits, leafy vegetables and milk powder) failed to improve B12 status in a large trial^{150,151}.

Use of widely available and regularly consumed foods as vehicles to deliver B12 is another preventive strategy. Fortification of wheat flour, bread, milk, breakfast cereal, nutrient bars, energy drinks and mineral water have been used with success^{152–154}. Probiotics have also been recently used with limited success to improve B12 status¹⁵⁵. Trials are essential in countries where B12 deficiency is a public health problem. Population-wide fortification of the diet with folic acid has been effective in reducing the incidence of neural tube defects in many countries⁹⁰. However, in individuals with a low intake of B12-consuming foods that are fortified with folic acid, data are emerging that excessively high levels of folate, occurring primarily as a result of additional folic acid consumption through supplement use, may aggravate their B12 deficiency^{98,99,156,157}. The mechanism underlying this adverse effect of high levels of folate on B12 status is not known.

Management

Treatment

In target groups that are at higher risk of B12 insufficiency, the goals are to prevent B12 deficiency in the first place and to treat such deficiency through repletion when it occurs. B12 deficiency is mainly caused by inadequate intake. Once B12 is repleted in these patients, B12 stores are generally well conserved because of an intact intrinsic factor-dependent absorptive mechanism and enterohepatic circulation of B12. This is not the case in those individuals who have some form of malabsorption caused by failure of the intrinsic factor-dependent conservation of biliary B12.

This fundamental difference in the capacity to conserve biliary B12 or not is an important determinant of both the rapidity of onset of depletion of body B12 stores and the severity of the B12 deficiency that supervenes when left untreated.

Severe clinical abnormalities should be treated intensively with B12, cyano-B12 or hydroxy-B12 (BOX 4). After an intramuscular injection of 1,000 µg of cyano-B12, about 150 µg is ultimately retained in the body, mainly through storage in the liver, although the variability in retention was large in the several original studies from which this figure was derived¹⁵⁸. Hydroxy-B12 is commonly used in Europe, often at intervals of 2–3 months, as it seems to have better retention than cyano-B12. There is no advantage to using the light-sensitive forms of cobalamin, such as methyl-B12 or adenosyl-B12, instead of the stable cyano or hydroxy forms, which are readily converted in the body into the coenzyme forms, methyl-B12 and adenosyl-B12 (REF. 159).

High-dose oral supplementation is an effective alternative to parenteral treatment. Radioactive B12 was used to show that 0.5–4% of an oral dose was absorbed; thus, high-dose tablets of 1,000 µg will deliver on average 5–40 µg of B12 (REF. 160). Randomized studies of daily high oral doses of 1,000–2,000 µg have shown equivalence or superiority to injected B12 (REFS 161–163). Oral doses of >500 µg daily have been necessary to correct MMA levels in elderly individuals, despite considerations that they have milder forms of B12 malabsorption^{164,165}.

Patients with true pernicious anaemia, lacking intrinsic factor, are unable to reabsorb the B12 lost in bile, which varies from 3 to 9 µg daily¹⁴¹. To maintain tissue stores, between 100 and 300 µg of B12 should therefore be retained monthly. The daily requirement for individuals without malabsorption has been set at 2.4 µg, although an intake of 4–7 µg resulted in lower serum MMA values¹⁶⁶. People who are B12 deficient due to low dietary intake will require loading with high-dose B12 to establish or restore tissue levels, after which time smaller supplements will suffice as conservation of biliary B12 is possible through enterohepatic recycling and the normally highly efficient reabsorption of biliary B12 (REF. 167). Detection of B12 malabsorption using the CibaSorb test can help to determine whether patients will respond to low-dose B12 supplements or whether patients will require treatment with pharmacological doses of the vitamin, either orally or by intramuscular injection¹³⁹. Establishment or restoration of adequate B12 stores is particularly important in pregnant and lactating women.

Severe neurological abnormalities should be treated aggressively with daily injections for a week and then weekly treatment until stabilized. Some patients request more-frequent injections, claiming subjective improvement in symptoms and mood. The basis for this is not understood, but as B12 is considered harmless with no defined tolerable upper intake level³⁸, there is no harm in a 'personalized' approach to meet the need of the individual patient.

Box 4 | Treatment of vitamin B₁₂ deficiency

Malabsorption

- Parenteral vitamin B₁₂ (B12) administration: 1,000 µg cyano-B12 or hydroxy-B12 intramuscular injection daily or every other day for 1 week followed by weekly injections up to 8 weeks then every 3–4 weeks*.
- Oral administration: high-dose cyano-B12 (2,000 µg) oral tablets daily until remission then 1,000–2,000 µg daily[‡].

Dietary deficiency

- Oral: consider a daily high dose to replace stores over 3–4 months then at least 6 µg daily.

Infants

- Parenteral: 250–1,000 µg intramuscular cyano-B12 or hydroxy-B12 daily, then weekly until recovery followed by oral 1–2 µg daily or B12-containing formulas, and treatment of the mother to correct breast milk.

*Hydroxocobalamin can be given every 2–3 months after the initial intensive treatment.

‡Lower doses of 600 µg have been shown to be adequate¹⁶⁵; elderly and post-gastric bypass patients should continue daily high-dose treatment.

Table 2 | Research needs relating to vitamin B₁₂ (B12) deficiency

Requirement	Current situation	Potential advantages
Better understanding of factors that influence B12 bioavailability	Bioavailability seems to vary widely, but the reasons are unclear	Improves prevention and management strategies
Improved knowledge about the role of the gut microbiota in B12 requirements	Studies suggest that variable degradation of B12 in the gut lumen might be influenced by the microbiota and that this might affect B12 bioavailability	Probiotics might be effective for prevention or treatment
Identification of obscure causes of B12 deficiency	In some patients who partially or fully respond to B12 treatment, testing does not identify the underlying metabolic defect	Improves prevention and management strategies
Better understanding of the relationship between folate and B12	Several epidemiological studies have found that high folate levels exacerbate the metabolic, haematological and neurological effects of B12 deficiency	Informs supplementation strategies, particularly in regions with both a high frequency of B12 deficiency and a population-based folic acid supplementation programme
Investigation of potential adverse effects of high B12 intake	No adverse effects have been identified, so there is no safe upper limit of B12 dose or level	Informs future large-scale, population fortification programmes
Improvements in the detection of B12 deficiency	Available tests have generally poor specificity and sensitivity	More-accurate identification of B12-deficient individuals
Development of reliable methods to identify B12 malabsorption	The current test (CobaSorb) does not discriminate between gastric and intestinal cause of B12 malabsorption and is only useful in patients who have not been treated with B12	Ability to confirm underlying absorptive defect and discriminate between gastric and ileal causes
Better understanding of the factors that determine variable manifestations of B12 deficiency	Why some patients with B12 deficiency show haematological features of B12 deficiency, whereas others show neurological features is not clear	Genetic differences for these variations in disease phenotype should be explored using whole-genome sequencing

Response to treatment

Megaloblastic anaemia caused by B12 deficiency responds to B12 treatment with reticulocytosis (increased numbers of immature red blood cells) in approximately 5 days and usually complete correction of the red blood cell count within 4–6 weeks¹⁴¹. If the patient is also iron deficient, then microcytic anaemia (anaemia with smaller than normal red cells) will be unmasked; other coexisting conditions such as chronic renal disease, inflammatory disorders, chronic liver disease or even coexisting myelodysplasia will also limit the response.

The response to neurological abnormalities is slower with the exception of mental symptoms such as emotional lability, paranoia and irritability, which may improve rapidly^{168,169}. There are occasional patients who have a transient exacerbation or new presentation of paraesthesia (a prickling sensation) or other complaints in the first week of treatment. The duration and severity of the neurological deficits before treatment generally predict the ultimate outcome¹⁶⁹. Paraesthesias without sensory loss or motor weakness are the most likely symptoms to completely correct. Imaging studies of the spinal cord show rapid correction of demyelination¹⁷⁰. If neurological symptoms progress after the patient has been satisfactorily treated, then they are not due to B12 deficiency and another cause should be sought.

Quality of life

Patients with true pernicious anaemia are at risk for other autoimmune disorders, particularly autoimmune thyroid disease. The chronic gastritis seen in patients with B12 malabsorption increases the risk of gastric carcinoid tumours and adenocarcinoma (FIG. 5), as well as malabsorption of other micronutrients; thus, iron deficiency and/or abdominal symptoms should be

evaluated promptly¹⁷¹. Patients with severe demyelinating central nervous system disease may have permanent impairments in proprioception, sensation and muscle weakness that impair quality of life¹⁶⁹. Generally, improvement will not continue after a year of adequate therapy. B12-deficient infants and young children may have permanent impairment in brain development and function¹⁷². A challenging aspect in the management of non-nutritional B12 deficiency is to ensure the lifelong treatment of the patient. Patients must be educated that the requirement for treatment is ongoing. An advantage of high-dose oral treatment in the United States is the easy over-the-counter availability of the supplements. Thus, patients are not dependent on health care providers for their replacement. However, patient or caregiver education is important to ensure continued compliance.

Outlook

The topic of B12 deficiency has attracted and still attracts considerable scientific attention, from physicians, scientists, the nutritional supplement and diagnostics industry and the public. Despite the body of knowledge on B12 deficiency that has accumulated, many key questions remain unanswered and many issues are unresolved (TABLE 2). The advent of ever more sophisticated and accurate analytical methods has refined the capacity to identify B12 status more precisely and to draw important distinctions between clinically evident B12 deficiency with its associated disease consequences and a preclinical state of B12 insufficiency that confers an increased risk or susceptibility for the development of certain disease states. Furthermore, from the public health perspective, understanding the conditions that predispose to B12 deficiency is key in order to implement the appropriate measures to prevent such deficiency in populations at risk.

Contrary to previous beliefs, B12 deficiency is not confined to elderly individuals, to white individuals and to individuals with intestinal malabsorption. Inadequacy of this nutrient, ranging from varying degrees of insufficiency to outright deficiency, has a wide prevalence and affects individuals of all ages, but most particularly infants, children, adolescents and women of reproductive age in populations in which dietary intake of B12-containing animal-derived foods is restricted. It is becoming increasingly clear that neurological consequences among the very young and the old are due, at least in part, to inadequate B12 status. There is clear evidence that low B12 status is a risk factor for cognitive decline and cerebral atrophy associated with ageing¹⁷³ and is associated with incident dementia⁹⁷. Moreover, vitamin supplements containing B12 delay these changes⁹⁶. Recent findings indicate that there is a progressive decrease in B12 levels in the brain across the lifespan, which parallels with an increased demand for antioxidants with age¹⁷⁴. At the other end of the age scale, in infancy, B12 deficiency is associated with neurological problems ranging from neuromuscular difficulty with swallowing to verbal development⁸⁷, and these problems have been ameliorated by administration of a

B12 supplement^{43,45}. Universal improvement of B12 status, therefore, seems to be a nutritional imperative with possibly profound beneficial effects on the nervous system, particularly at the bookends of life.

One of the most pressing and enduring questions about B12 deficiency is how and to what extent it is influenced by the supply, and particularly excesses of its closely affiliated vitamin, folate. Another enigma in B12 deficiency is the often widely differing manifestations among patients with regard to the dominance of either haematological or neurological complications. The spectrum of the B12-deficient phenotype is broad^{1,4-6}, yet the reasons for this remain unknown. It is possible that genetic factors or nutrient–nutrient interactions may explain these differences in susceptibility¹⁷⁵⁻¹⁷⁷.

Much has been learned about B12 since its isolation and characterization almost 70 years ago. As the pace of scientific progress continues to accelerate, it is likely that there will be answers to most of the questions posed in this Primer, but other scientific questions are bound to arise. Above all, the anticipation that the global burden of B12 deficiency will be considerably alleviated is an outcome that is both reasonable and desirable.

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Author contributions

Introduction (R.G.); Epidemiology (L.H.A., A.B., A.M.M., A.-L.B.-M., J.W.M. and P.M.U.); Mechanisms/pathophysiology (J.-L.G. and B.-H.T.); Diagnosis, screening and prevention (E.N. and C.Y.); Management (S.S.); Quality of life (S.S.); Outlook (R.G.); Overview of Primer (R.G.).

Competing interests

R.G. has previously served on speakers' bureaus and as a consultant for Emisphere Technologies. J.W.M. has served on a scientific steering committee for Emisphere Technologies. A.M.M. received an honorarium as a speaker at the Abbott Transformation Forum, Manchester, UK. S.S. indirectly benefits from the activities of a company formed by the University of Colorado aimed at measuring vitamin B₁₂-related metabolites. Otherwise she does not have any conflict of interest. All other authors declare no competing interests.

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➔ Vitamin B₁₂ (B12) — also known as cobalamin — has an important role as a cofactor in many cellular processes, including DNA synthesis and methylation, and mitochondrial metabolism. Deficiency is associated with various symptoms, of which the haematological (mainly anaemia) and neurological (such as sensory and motor disturbances, ataxia and psychiatric disorders) manifestations are the most characteristic.

MECHANISMS

! Autoimmune destruction of parietal cells and the consequent absence of intrinsic factor causes the classic manifestation of B12 deficiency — pernicious anaemia

PREVENTION

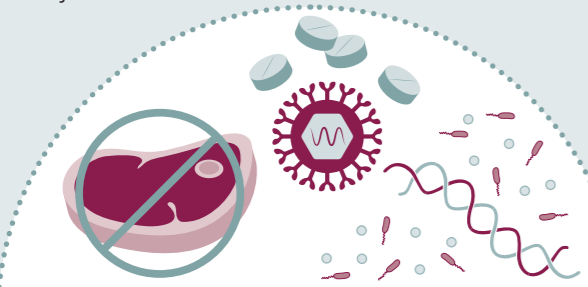
B12 deficiency is an emerging public health concern, especially in developing countries and in populations at risk. In addition, correction of B12 levels

can lower increased homocysteine levels in populations receiving folic-acid-fortified products or in individuals using folic acid

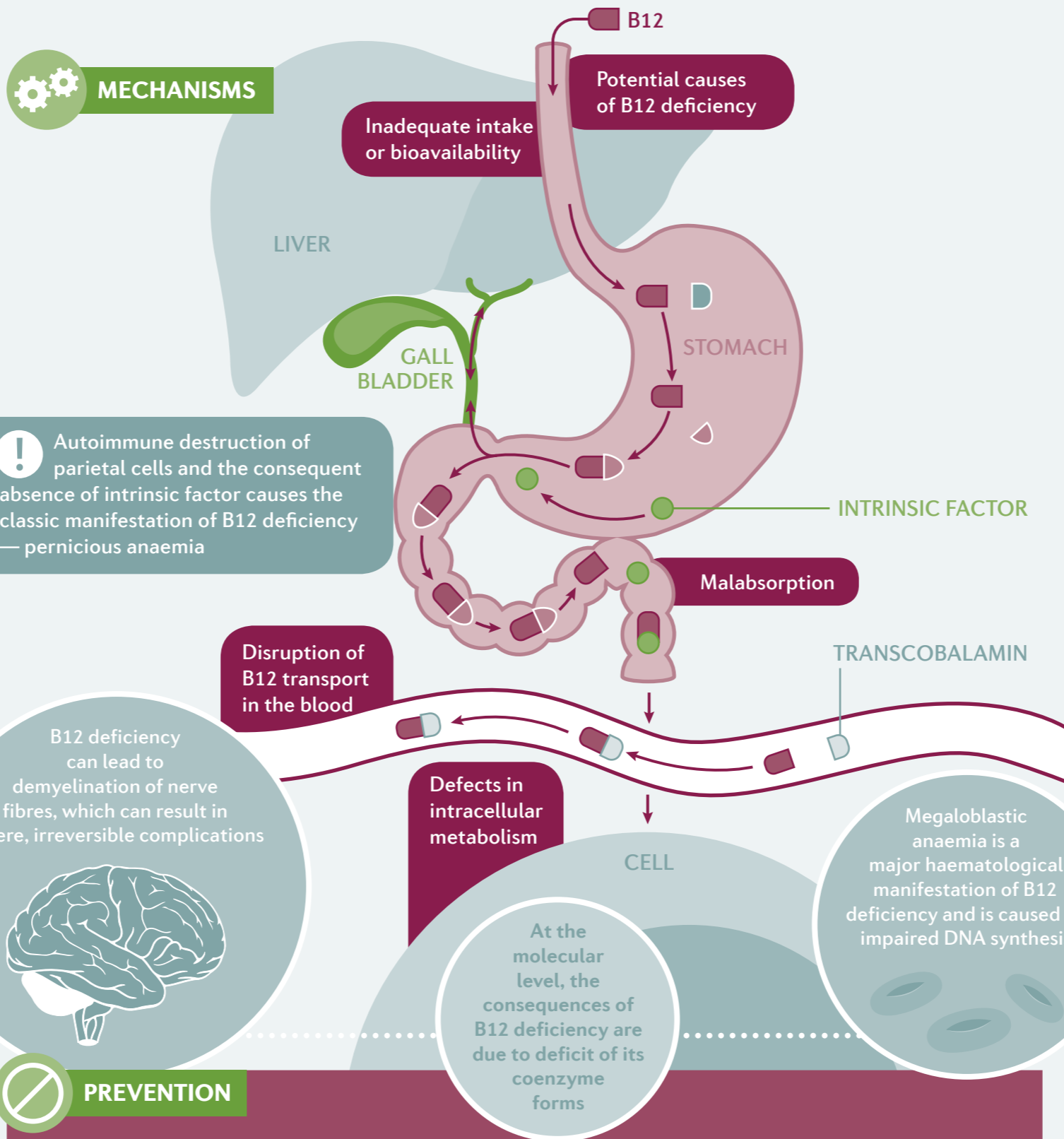
supplements. Low B12 levels and increased homocysteine levels have been associated with cognitive impairment in elderly individuals.

EPIDEMIOLOGY

Clinical B12 deficiency associated with either severe anaemia or neurological manifestations or both is uncommon (1–2% of individuals >60 years of age), but subclinical deficiency affects 3–26% of the general population in the United States, depending on the biomarker cut-off levels used. Prevalence is much higher in South America, Asia and Africa. Demand and availability of B12 varies throughout life, making specific subpopulations vulnerable to deficiency, especially infants, children, women who are pregnant or breastfeeding and elderly individuals.

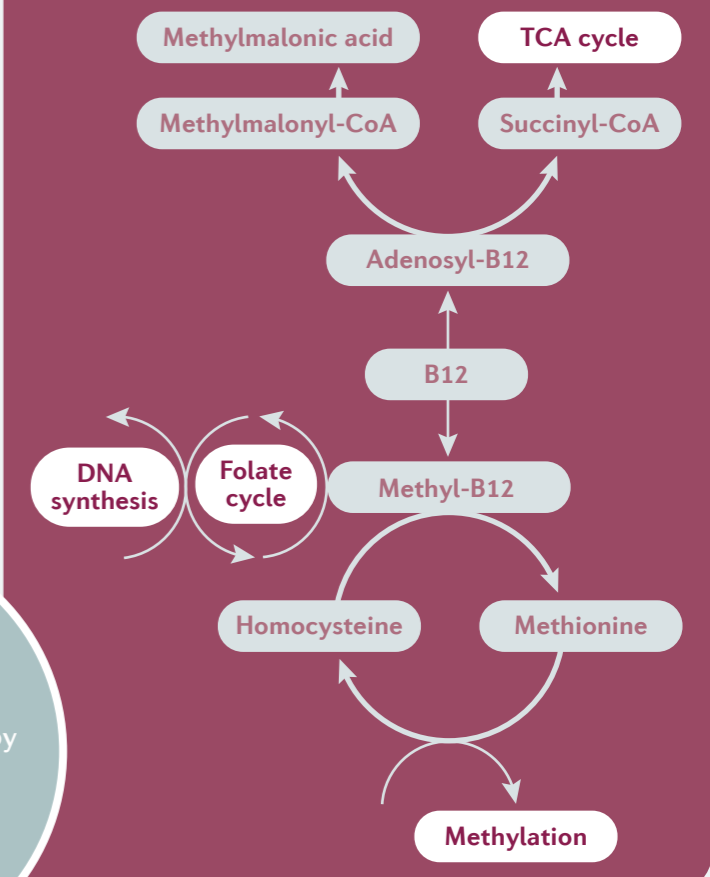


! Risk factors for developing B12 deficiency are failure of the intrinsic factor–B12 absorption pathway, inadequate intake of B12-containing foods (mainly meat), genetic factors, gastrointestinal infection or surgery, long-term use of drugs (such as gastric H⁺/K⁺ inhibitors or histamine H₂ receptor antagonists) and concomitant illnesses such as HIV infection and tuberculosis.



DIAGNOSIS

As the clinical presentation of B12 is so varied, a combination of blood biomarkers is often used for diagnosis, despite limitations. These markers include low total B12 levels and transcobalamin-bound B12 levels (that is, the active form of B12), and increased homocysteine and methylmalonic acid levels. However, the exact cut-off levels to classify normal, subclinical or clinical B12 deficiency are not firmly established.



MANAGEMENT Rx

Depending on the underlying cause, B12 repletion generally involves oral (if the cause is inadequate intake) or parenteral (if the cause involves malabsorption) B12 administration. After a high initial load, the dose is usually reduced.